THE
BIOLOGICAL BULLETIN

PUBLISHED BY
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THE BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

THE MARINE BIOLOGICAL LABORATORY

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II. ACT OF INCORPORATION

No. 3170

Commonwealth of Massachusetts

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips, and B. H. Van Vleck have associated themselves

with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, HENRY B. PIERCE, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our Lord One Thousand Eight Hundred and Eighty-Eight.

[SEAL]

HENRY B. PIERCE,
Secretary of the Commonwealth.

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. The members of the Corporation shall consist of persons elected by the Board of Trustees.

II. The officers of the Corporation shall consist of a President, Vice President, Director, Treasurer, and Clerk.

III. The Annual Meeting of the members shall be held on the Friday following the second Tuesday in August in each year at the Laboratory in Woods Hole, Massachusetts, at 9:30 A.M., and at such meeting the members shall choose by ballot a Treasurer and a Clerk to serve one year, and eight Trustees to serve four years, and shall transact such other business as may properly come before the meeting. Special meetings of the members may be called by the Trustees to be held at such time and place as may be designated.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

VI. Inasmuch as the time and place of the Annual Meeting of members are fixed by these By-laws, no notice of the Annual Meeting need be given. Notice of any special meeting of members, however, shall be given by the Clerk by mailing notice of the time and place and purpose of such meeting, at least fifteen (15) days before such meeting, to each member at his or her address as shown on the records of the Corporation.

VII. The Annual Meeting of the Trustees shall be held promptly after the Annual Meeting of the Corporation at the Laboratory in Woods Hole, Mass. Special meetings of the Trustees shall be called by the President, or by any seven Trustees, to be held at

such time and place as may be designated, and the Secretary shall give notice thereof by written or printed notice, mailed to each Trustee at his address as shown on the records of the Corporation, at least one (1) week before the meeting. At such special meeting only matters stated in the notice shall be considered. Seven Trustees of those eligible to vote shall constitute a quorum for the transaction of business at any meeting.

VIII. There shall be three groups of Trustees:

(A) Thirty-two Trustees chosen by the Corporation, divided into four classes, each to serve four years. After having served two consecutive terms of four years each, Trustees are ineligible for re-election until a year has elapsed. In addition, there shall be two groups of Trustees as follows:

(B) Trustees ex officio, who shall be the President and Vice President of the Corporation, the Director of the Laboratory, the Associate Director, the Treasurer, and the Clerk:

(C) Trustees Emeriti, who shall be elected from *present* or *former* Trustees by the Corporation. Any regular Trustee who has attained the age of seventy years shall continue to serve as Trustee until the next Annual Meeting of the Corporation, whereupon his office as regular Trustee shall become vacant and be filled by election by the Corporation and he shall become eligible for election as Trustee *Emeritus* for life. The Trustees *ex officio* and *Emeritus* shall have all the rights of the Trustees except that Trustees *Emeritus* shall not have the right to vote.

The Trustees and officers shall hold their respective offices until their successors are chosen and have qualified in their stead.

IX. The Trustees shall have the control and management of the affairs of the Corporation; they shall elect a President of the Corporation who shall also be Chairman of the Board of Trustees and who shall be elected for a term of five years and shall serve until his successor is selected and qualified; and shall also elect a Vice President of the Corporation who shall also be the Vice Chairman of the Board of Trustees and who shall be selected for a term of five years and shall serve until his successor is selected and qualified; they shall appoint a Director of the Laboratory; and they may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. The Board of Trustees shall have the power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

X. The Associates of the Marine Biological Laboratory shall be an unincorporated group of persons (including associations and corporations) interested in the Laboratory and shall be organized and operated under the general supervision and authority of the Trustees.

XI. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be disposed of in such manner and upon such terms as shall be determined by the affirmative vote of two-thirds of the Board of Trustees.

XII. The account of the Treasurer shall be audited annually by a certified public accountant.

XIII. These By-laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-laws will be acted upon.

IV. REPORT OF THE DIRECTOR

TO THE TRUSTEES OF THE MARINE BIOLOGICAL LABORATORY:

Gentlemen:

I submit herewith the report of the seventieth session of the Marine Biological Laboratory.

During the past year the Laboratory made significant progress in rehabilitating some of its research space and facilities and also funds were obtained for a new research building and additional housing.

1. Crane Building

The Officers of the Laboratory held several conferences during the winter (1957) with representatives of our architectural firm, Coolidge, Shepley, Richardson and Abbott, to develop plans for the rehabilitation of the Crane Building under the National Science Foundation Grant of $415,000. Planning was completed early in the summer; the contracts and subcontracts were let in August. A detailed schedule of the operation was developed by the general contractor, the building was evacuated by the Laboratory immediately after Labor Day and the remodeling started. The schedule called for the completion of the job by May 1, 1958. Present indications are that the work will be completed on time, and that the equipment can be moved in for occupancy by the investigators not later than June 1.

Out of this remodeling the Laboratory will have an essentially new building with facilities for any type of research in biology and the cognate sciences. The rearrangement of the standard facilities within the individual laboratories will result in a much more efficient use of available space.

2. New Research Laboratory

In 1938 it was recommended by an *ad hoc* committee set up to formulate policy on the future development of the Marine Biological Laboratory that the wooden buildings should be replaced by a modern brick laboratory building. At the Annual Meeting of the Board of Trustees, August 16, 1957, the Officers of the Laboratory were authorized to seek funds to implement this recommendation. Applications were made to the Rockefeller Foundation, the National Science Foundation and the Public Health Service for the necessary funds. Early in December the Laboratory was notified of a grant from the Rockefeller Foundation of $738,500, providing one-half the necessary funds. Subsequently, grants were obtained from the National Science Foundation and the Public Health Service which shared equally in providing the other half of the cost of the new building and its equipment. Planning for the new building is already under way, construction to start in the fall of 1959 with occupancy planned for the spring of 1961. It will be a three-story and basement building, almost entirely devoted to research and research service laboratories.

3. Housing

The 1938 *ad hoc* Committee on the Development of the Laboratory also expressed its concern with the problems of housing and adequate care of the large number of persons attracted to the community by reason of their Laboratory activities. It was pointed out that the housing needs have, from the beginning, been recognized as one of the primary responsibilities of the Laboratory and that the arrangements then existing were not adequate. Since that report, three frame houses in the immediate vicinity of the Laboratory have been acquired and converted to dormitory use. Since World War II there has developed an acute shortage of housing for younger married investigators with children. Toward the end of the year (1957) the Laboratory made application to the National Science Foundation for a grant of $175,000 to erect 25 housekeeping cottages on the Laboratory's Devils Lane Property. Favorable action was taken by the National Science Foundation on this grant request. Plans have been developed for these cottages which will be erected for 1959 occupancy.

Also, the Board of Trustees voted to discontinue any further sale of lots from the Devils Lane Property so that the Laboratory will retain title to the remaining 75 acres for future Laboratory use, either housing or scientific.

4. Grants, Contracts and Contributions

The total income to the Laboratory from these sources of support amounted to $210,000 in 1957. This represents 32% of the total income and consists of the following accounts:

American Cancer Soc.—R-7G—Fundamental Studies in Radiobiology	$ 6,600.00
A.E.C.—1343—Program of Research on the Physiology of Marine Organisms Using Radioisotopes	9,545.00
N.I.H.—4359—Biological Research on the Morphology, Ecology, Physiology, Biochemistry and Biophysics of Marine Organisms	40,000.00
N.I.H.—5143—Training Program in Nerve-Muscle Physiology	40,342.00
National Science Found.—G2142—Funds for Biological Research	25,000.00
National Science Found.—G3608—Optical Equipment	11,500.00
National Science Found.—G3987—Centrifuge Equipment	10,000.00
O.N.R.—1497—Studies in Marine Biology	15,000.00
O.N.R.—09701—Studies on Isolated Nerve Fibers	7,670.00
O.N.R.—09702—Studies in Ecology	5,268.00
M.B.L. Associates	3,481.00
Abbott Laboratories	1,000.00
American Philosophical Society	2,500.00
Ciba Pharmaceutical Products, Inc.	1,000.00
Eli Lilly Company	5,000.00
Merck and Company, Inc.	1,000.00
Rockefeller Foundation	20,000.00
Schering Corporation	1,000.00
Smith, Kline, and French Foundation	3,000.00
The Upjohn Company	1,000.00
	$209,906.00

5. Boats

Late in the year the Laboratory contracted with the Riverside Boat Company of Newcastle, Maine, for two 24-foot boats for trap work and inshore collecting. These boats will replace the old *Sagitta* and *Tern*, both of which, after years of service, outlived their usefulness. The new boats are to be delivered in May (1958).

Respectfully submitted,
PHILIP B. ARMSTRONG,
Director

MEMORIAL

BENJAMIN M. DUGGAR
by
Wm. Randolph Taylor

Benjamin Minge Duggar, late Emeritus Trustee of this Laboratory, died 10 Sept. 1956 in New Haven, Conn. Dr. Duggar was born in Gallion, Alabama in 1872. His early education in private schools was followed by studies in civil engineering at the University of Alabama and the Mississippi Agricultural and Mechanical College, where his interest shifted to botany, and he received his B.S. in 1891. He then completed his work for the M.S. at Alabama Polytechnic, but, going to Harvard University, qualified there for the A.B. and A.M. degrees, transferring to Cornell University where he received the doctorate in 1898, completing his formal studies. He subsequently worked in several laboratories in Europe, further widening his experience. His government and academic appointments were numerous, but four institutions claimed his services as full Professor before retirement: first the University of Missouri, then Cornell, and then for much longer periods Washington University and the University of Wisconsin.

His distinguished academic career was marked by a happy combination of physiology and pathology, in both of which fields he contributed notably in research and produced textbooks of exceptional merit, that in plant pathology remaining useful to this day. He contributed his share, also, to one of the most successful American elementary botanical text-books ever produced, that prepared by the Wisconsin group and still in use. During his period at Wisconsin the Department of Botany strengthened its position as one of the most notable in the country. His researches covered a considerable range of endeavor, but those on virus diseases, particularly the mosaic disease of tobacco, are most often remembered.

Dr. Duggar does not seem first to have appeared at the Marine Biological Laboratory as a student or as an investigator, as is commonly the case. In 1909 he was appointed to what was termed the "Research Staff" in botany, while Professor of Physiology at Cornell. In 1911 the course in botany was divided, the first three weeks dealing with the algae, the second with "The Physiology and Ecology of Marine, Strand and Bog Vegetation" with Lewis Knudsen, also from Cornell, as his associate. Knudsen was replaced in 1912 by W. J. Robbins, best known as the Director of the New York Botanical Garden, but the course was dropped in 1915. "Investigation Staff" replaced the old term for the advisory group, and Dr. Duggar served Botany on this board from 1926 to 1941. He was elected to the Corporation in 1911 and to the Trustees of the Laboratory in 1928, retiring Emeritus in 1944. During all these years he was frequently in residence through the summer, and always helpful to those at the Laboratory whose enquiries fell within his field of interest.

The discernment shown by Dr. Duggar respecting the affairs of the Marine Biological Laboratory was appreciated by other institutions, and he served as Trustee of the Bermuda Biological Station 1933–1937, and of the Woods Hole Oceanographic Institute from its inception in 1931 until 1938. Honorary degrees were bestowed on him by Washington University, the University of Missouri and the University of Wisconsin; he was elected to the most distinguished of our learned and professional societies.

On retirement from Wisconsin Dr. Duggar promptly joined the research staff of the Lederle Laboratories of the American Cyanamid Company, and settled down to research on the discovery, production and evaluation of antibiotics from various Actinomycete bacteria. All reports from the company describe with admiration his quiet industry and the keen mind he placed most generously at the disposal of his fellow workers. His most spectacular success was in the discovery in 1945 of Aureomycin, a very effective antibiotic, which has gone into extensive commercial production. He continued active in research until his final illness.

Dr. Duggar lost his first wife in 1922; his second wife, several children and grandchildren survive him. To them we wish to express our appreciation of his many contributions to science and our sympathy in the loss they have suffered.

Mr. President, I move that a copy of this memorial be placed in the minutes of this meeting, and that a copy be sent to Mrs. Duggar.

MEMORIAL

E. S. G. Barron

by

H. B. Steinbach

E. S. G. Barron, "Achito" to many, died this summer and is buried in the cemetery at his beloved Woods Hole. His scientific studies achieved world-wide recognition as did the charming personality of the man responsible for them.

While Barron was truly a scientist of the world, his ties to the Marine Biological Laboratory were strong and his affection for the area was great. He was elected a member of the Corporation in 1933, a trustee in 1949 and again in 1952. He served as instructor in the Physiology Course from 1945 until 1948 when he assumed the headship for a five-year period. Under his guidance the course continued its strong development and became especially well known on the international scene. He was largely instrumental in obtaining much of the special equipment that is now in use. He conducted the special session of the course in honor of his revered teacher, Leonor Michaelis, and edited the volume "Modern Trends in Physiology and Biochemistry" which carried the fame of the MBL even farther than before.

For the past several years Barron found it necessary to give up his attendance here to carry out a labor of love dear to his heart, spending his summers in teaching and consulting in South America as his contribution to the advancement of science in those areas, especially the country of his birth, Peru.

While he was perhaps best known for his studies on oxidative mechanisms, Achito's interests and activities were very broad indeed, ranging from a classical work on bilirubinemia to the effects of ionizing radiations on crystalline proteins. However he was preëminently a biologist and, in his mind, all his studies were fundamentally directed at understanding cellular oxidations and their regulation. Shortly before his death, his plans for future work were keyed largely to a comprehensive comparative study of cellular oxidations with the hope of finding critical keys to physiological regulations.

Barron was born in Huari, Peru, in 1898. Following two years in France he came

to this country in 1927, first as a Rockefeller Fellow and then as instructor in Johns Hopkins University. In 1930 he moved to the University of Chicago, his University until his death. During World War II, he did scientific work for both the AEC and the Medical Division of the Chemical Warfare Service. He was especially well fitted to carry out the important studies on effects of ionizing radiations and the biological actions of nitrogen mustards and related compounds.

Achito was a remarkable teacher even though his position at Chicago did not involve conducting formal classes. He had a keen and incisive sense of humor and a fine critical attitude towards science. Many have benefitted from his wisdom and have been delighted with his conversation. He had a strong sense of the necessity for training minds in intellectual pursuits. This led him to his fruitful efforts in the Woods Hole course and in the training programs in South America. When he purchased his new home in Chicago some years ago, his greatest delight was that he had a large pleasant room with a big blackboard. Here he could invite his students and colleagues for seminars and discussions and here many of the ideas for experiments by himself and collaborators were born.

Achito, his wife Cora, and his son Richard constituted a family it was a pleasure and privilege to know. And while we are expressing our gratitude to Achito for his many contributions to us, we must include his wife and son for providing the setting for such a fruitful career.

As an experimentalist, as a teacher, as one who travelled widely and spread the traditions of science and inspired the young, Barron was at the height of his activity when he became ill and died. At such a time it is not trite to say that a man's death is untimely.

MEMORIAL

Robert Chambers

by

B. W. Zweifach and G. H. A. Clowes

With the death of Robert Chambers at the age of 75 on July 22nd, the Marine Biological Laboratory lost one of the most illustrious members of its old guard—marking as it were the passing of an era in which microscopy as a fine art was utilized to its fullest extent for the study of cellular behavior and protoplasmic structure. Chambers' associations here in Woods Hole were long and deep-rooted. He first came to the M. B. L. in the summer of 1911 as a graduate investigator and in 1912 was on the teaching staff in Zoology and Embryology—a course in those days associated with such names as Calkins, Lillie, Conklin, Morgan and Wilson. By 1914 it became apparent that Chambers' interests were not along the lines of conventional zoology and he was thereafter listed in the annual reports of the M. B. L. as an investigator in Physiology—an indication that the science of cellular physiology had come of age.

By training Robert Chambers was a histologist and embryologist. He was born and raised in Turkey, where his parents resided as missionaries. The rough, harsh life during his formative years left an indelible imprint on his makeup and was to a considerable extent responsible for his great compassion for the underdog and his willingness to champion humanitarian causes. It was at Roberts College that his interest in nature was crystallized and his future course indicated. Later, under the aegis of Hertwig and Goldschmidt in Munich, where he received his Ph.D in 1908, Chambers was indoctrinated into the field of histophysiology and developed a keen interest in basic embryology. He returned to Canada, the early home of the Chambers family, and eventually joined Cornell Medical College in 1915. These were his most fruitful years—his outstanding contributions in large part derived from his ingenious researches at Woods Hole. His laboratory

here in Room 328, in association with the Eli Lilly group, was a beehive of activity where Chambers' dynamic personality infused all who worked with him. Few could keep pace with his amazing physical stamina and drive. At an early age Robert Chambers became virtually a legendary figure, not only because of his scientific stature but because of the anecdotes which grew up around his prodigious unconcern for practical matters. There are many here who knew him during these inspiring years as a most attractive and congenial personality. Woods Hole was the center of the social and scientific life of Robert and Bertha Chambers. They practically raised their four sons at Bobtuckett Cottage and many of the delightful and entertaining experiences of the Chambers family have attained the stature of local folklore. Robert might be found at almost any time, day or night, in his M. B. L. laboratory and the Chambers family regarded the remainder of the year as an unavoidable intrusion into the Woods Hole continuum.

In 1928 he transferred from the Anatomy Department at Cornell to the Department of Biology at New York University, where he maintained until his retirement in 1949 a research center which attracted students from every country of Europe, from Asia, and from South America, many of whom are today outstanding figures in scientific research.

The magnitude of Robert Chambers' contribution becomes all the more impressive when it is considered that he published over 230 scientific articles, bearing in mind the fact that writing was extremely burdensome for Chambers. A great deal of what he did, he left for others to put into words. He unflaggingly, to the point of self-denial, gave his time and counsel to a never-ending stream of students, associates, cronies and visitors. Time was a meaningless entity to him.

In 1912, at the M. B. L. seminar sessions, Chambers was greatly stimulated by a lecture in which G. L. Kite showed that it was possible to interfere with the development of marine ova with glass microtools. In retrospect, we can see that this event proved to be the turning point in his scientific career. The potentialities of this approach appealed so much to Chambers that he developed and applied the microsurgical technique extensively, his name becoming synonymous with the micromanipulative method. In his early work, principally at Woods Hole, he clearly showed the importance of sol-gel transformations in relation to aster formation and cell division. There followed the beautiful demonstrations, accompanied by motion pictures, of the capacity of the cytoplasm and cell surface to recover from various forms of microinjury in the presence of the proper ionic environment. He made the earliest measurements of the pH of the cytoplasm in intact cells, using indicator dyes. His enthusiasm was such that every aspect of cellular behavior intrigued him, the cohesion of blastomeres in developing embryos, the action of salts on protoplasm, the nature of vital staining, the interfacial tension at protoplasmic surfaces, the acid of injury, etc.

Later, Chambers combined tissue culture with microtechniques. Especially noteworthy were his studies on malignant cells, the secretory activity of kidney tubules and chemotactic phenomena. During World War II, he devoted a goodly part of his energies to studies on capillary permeability and to the vascular sequelae of experimental shock. New and important concepts of circulatory homeostasis were originated.

Numerous honors were bestowed upon Chambers. As early as 1926 he gave his first Harvey lecture on the living cell. During this period he received the Traill medal from the Linnean Society of London, the John Scott medal from the City of Philadelphia, the medal of L'Academie Nationale de Médecine of Paris, was made a Fellow of the Royal Microscopical Society of England, and was given an honorary LL.D. from Queens University. He was active in the affairs of many societies, having been a Trustee of the Marine Biological Laboratory, a member of the Board of Directors of the Long Island Biological Association, President of the American Society of Zoologists, the Harvey Society, the Union of American Biological Sciences, and vice-president of the American Association of Anatomists.

When one attempts to give an account of a man's life in a few hundred words, the impossibility of the task becomes increasingly apparent. In the case of Robert Chambers, his human qualities transcended even his outstanding scientific achievements. His later years were saddened by the loss of his oldest son, Robert, in World War II, and by the protracted illness and death of his wife Bertha. It would be a mere platitude to say that we shall miss him, but we hope that the imprint of his indomitable spirit will live on in those of us who were fortunate enough to know him and to contribute some small part to the fruits of his labor.

1. The Staff, 1957

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A. C. Redfield, Woods Hole Oceanographic Institution

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John B. Buck, Senior Biologist, National Institutes of Health
Clark P. Read, Associate Professor, School of Hygiene and Public Health, Johns Hopkins University
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Morris Rockstein, Associate Professor of Physiology, New York University College of Medicine
Cadet Hand, Assistant Professor of Zoology, University of California, Berkeley
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M. Sussman, Associate Professor of Biological Sciences, Northwestern University
J. P. Trinkaus, Associate Professor of Zoology, Yale University
P. B. Weisz, Associate Professor of Zoology, Brown University
E. Zwilling, Program Director, National Science Foundation (on leave from University of Connecticut)

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KARUSH, FRED, Associate Professor of Immunology, University of Pennsylvania
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LONDON, IRVING M., Professor and Chairman, Department of Medicine, Albert Einstein College of Medicine
LOVE, LOIS H., Research Associate, National Research Council
MCDONALD, SISTER ELIZABETH, Chairman, Department of Biology, College of Mt. St. Joseph
MOORE, GEORGE M., Professor and Chairman of Zoology, University of New Hampshire
NOVIKOFF, ALEX B., Research Associate Professor of Pathology, Albert Einstein College of Medicine

PICK, JOSEPH, Professor of Anatomy, New York University-Bellevue Medical Center
ROOT, WALTER S., Professor of Physiology, College of Physicians and Surgeons
ROSE, S. MERYL, Professor of Zoology, University of Illinois
SCHLESINGER, R. WALTER, Director, Department of Microbiology, St. Louis University School of Medicine
SCOTT, ALLAN, Professor of Biology and Chairman of Department, Colby College
SHERMAN, FRANK E., Assistant Professor of Pathology, University of Pittsburgh
STEINHARDT, JACINTO, Director, Operations Evaluation Group, Massachusetts Institute of Technology
SULKIN, S. EDWARD, Professor and Chairman, Department of Microbiology, University of Texas, Southwestern Medical School
WAGNER, ROBERT R., Assistant Professor of Medicine, Johns Hopkins University School of Medicine
WARNER, ROBERT C., Associate Professor of Biochemistry, New York University College of Medicine
WHEELER, GEORGE E., Instructor of Biology, Brooklyn College
WHITEHOUSE, MICHAEL W., Instructor of Biochemistry, University of Pennsylvania School of Medicine
YNTEMA, CHESTER L., Professor of Anatomy, State University of New York College of Medicine
ZORZOLI, ANITA, Associate Professor of Physiology, Vassar College

LALOR FELLOWS, 1957

BACON, DONALD, Yale Medical School
BISHOP, NORMAN, University of Chicago
BRYANT, S. H., University of Cincinnati
BÜCKMANN, DETLEF, Zoologisches Institut, Mainz, Germany
BURGEN, A. S. V., McGill University
EDWARDS, CHARLES, Johns Hopkins University
ENGLE, RALPH, Cornell University Medical College
LORAND, L., Northwestern University
LINDBERG, OLOV, Wenner-Grens Institute, Sweden
LUBIN, MARTIN, Harvard Medical School
SCHULMAN, MARTIN, State University of New York, College of Medicine at Syracuse
STEPHENSON, W. K., Earlham College
WHITEHOUSE, MICHAEL, University of Pennsylvania School of Medicine
WILSON, T. HASTINGS, Washington University School of Medicine
WOODS, KENNETH, Cornell University Medical School

Lillie Fellow, 1957

NIU, MAN-CHIANG, Rockefeller Institute for Medical Research

Students, 1957

BOTANY

ABELES, FRED B., Cornell University
ARNOLD, ELIZABETH I., University of Rochester
ARONSON, FLORA P., Brooklyn College
BOUCK, GEORGE B., Columbia University
COOK, PHILIP W., University of Vermont
CZELUSNIAK, MARILYN M., Smith College
FRANKEL, JOSEPH, Yale University
HERSKOWITZ, JULIA, Antioch College
KEELER, CARL R., JR., Northwestern University
KLEPPER, ELIZABETH, Vanderbilt University

KUENZLER, EDWARD J., University of Georgia
MORELAND, RALPH E., JR., Indiana University
MUSCHIO, HENRY M., Fordham University
PAIK, HYANGJU, Wellesley College
PAOLI, GISELA, Chatham College
PARKER, BRUCE C., Yale University
PROTA, CARL D., Fordham University
RICE, ELEANOR, Wheaton College
TEWS, LEONARD C., Indiana University
WALSER, STEPHANIE L., Radcliffe College

EMBRYOLOGY

ARKLESS, RICHARD, University of Pennsylvania Medical School
CASTON, J. DOUGLAS, University of North Carolina
GOERINGER, GERALD C., Johns Hopkins University
GRIFFIN, JOE LEE, Princeton University
HANKS, JAMES E., University of New Hampshire
HERSH, GEORGE L., University of California
KARAKASHIAN, STEPHEN J., Drew University
KERR, NORMAN S., Northwestern University
KESSEL, RICHARD G., State University of Iowa
KIRCHEN, ROBERT V., Columbia University
KRAM, FLEURETTE L., Northwestern University
LOVE, DAVID S., University of Colorado
LOWE, JANET M., University of Minnesota
MATHIESEN, GEORGE C., Harvard University
MELLON, DEFOREST, JR., Yale University
NELSON, SHIRLEY, Northwestern University
ROSEWATER, JOSEPH, Harvard University
SPARANO, BENJAMIN M., Fordham University
TALBOT, WILLIAM H., Rockefeller Institute
TYSON, GRETA E., University of New Hampshire
VAN DYK, N. JOANNE, University of New Hampshire
WALCOTT, CHARLES, Cornell University
WATKINS, MARGARET J., University of Minnesota
WHITE, JEAN ANN, Mount Holyoke College
WYLIE, RICHARD M., Harvard University

PHYSIOLOGY

CLARK, ALVIN JOHN, Harvard University
COX, RODY P., University of Pennsylvania
DAVIDSON, MORTON, Bellevue Medical College
DUBNAU, DAVID A., Columbia University
ERWIN, JOSEPH A., Syracuse University
FAHN, STANLEY, University of California School of Medicine
FELIX, MARIE D., Cornell University Medical School
HAFT, DAVID E., University of Rochester School of Medicine
HALPEREN, SIDNEY, University of Texas
KAHLBROCK, MARGIT, Columbia University
KIRSCH, JACK F., Rockefeller Institute
MCCLUSKEY, ROBERT T., New York University-Bellevue Medical Center
MAZUR, PETER, Princeton University
MEDINA, HEITOR S., Inst. de Biolojia—Curitiba, Paroni, Brazil
MINDICH, LEONARD E., Rockefeller Institute
NAGLER, ARNOLD L., Bellevue Medical School
OTERO, LUIS R., University of Puerto Rico
RABINOWITZ, LAWRENCE, University of California
RAWITSCHER, ERIKA, American Museum of Natural History

ROBERTS, PATRICIA R., Duke University
SCHNEIDER, JOHN H., University of Wisconsin
SIGER, ALVIN, Johns Hopkins University
STERN, DANIEL N., Albert Einstein College of Medicine
STONE, NANCY J., Columbia University
TAKEUCHI, IKUO, Princeton University
WEEKS, BOYD M., University of California
WILLIAMS, FRANK ROBERT, Oberlin College
WILLIAMS, FREDERICK M., Yale University
WILLIS, JOHN S., Harvard University

INVERTEBRATE ZOOLOGY

ASHER, DAVID M., Harvard University
AUGENFELD, JOHN M., University of Wisconsin
BECKER, JOYCE E., Evansville College
BRANNING, ARLEEN, City College of New York
BRAVERMAN, MAXWELL H., University of Illinois
CAMP, DONALD B. M., Acadia University
CLARKE, ARTHUR H., JR., Cornell University
COLEMAN, CHASE, Vassar College
CONCANNON, BRO. JOSEPH, St. John's University
COOPER, MADELINE, American Museum of Natural History
COOPER, KENNETH K., American Museum of Natural History
CROWELL, JANE, Oberlin College
DIAMOND, JARED M., Harvard University
DOBBEN, PHYLLIS A., Rocky River 16, Ohio
DOBBS, HARRY D., Wofford College
EGLOFF, DAVID A., Amherst College
GFELLER, SISTER MARION D., Marquette University
GUZE, CAROL D., Washington University
HAFENER, PAUL A., JR., Franklin and Marshall College
HECHTEL, GEORGE J., Yale University
HILD, DAVID H., Wesleyan University
HORVATH, NANCY, 10121 S. Parnell Avenue, Chicago 38, Illinois
HORWITZ, JUDITH, Radcliffe College
ISAAC, DONALD E., University of California
JENSEN, DONALD DALE, Yale University
JOHNSON, B. THOMAS, University of California
JORDAN, ELKE, Goucher College
KAUFMAN, JOHN H., University of California
KRASSNER, STUART, Johns Hopkins University
LANE, ROSEMARY M., Dalhousie University
LEISY, ELSA, University of California
LONGACRE, HARRIETTE, Mount Holyoke College
LORENZO, MICHAEL A., St. Louis University
LOWE, MILDRED E., Tulane University
MCMANUS, LAWRENCE ROBERT, Cornell University
MENAKER, MICHAEL, Princeton University
NEWBERRY, ANDREW TODD, Stanford University
POULSON, THOMAS L., University of Michigan
PRAGER, JAN C., University of Cincinnati
REESE, ERNST S., University of California
ROOT, RICHARD B., University of Michigan
ROSS, SHIRLEY E., Washington University
SHERMAN, IRWIN W., City College of New York
SMITH, S. CLARKE, Wabash College
SMITH, SUSAN, Earlham College
THOMPSON, JANE F., University of Massachusetts
THOMPSON, MARTHA JANE, Oberlin College

TROTTER, NANCY L., Brown University
VITOLS, ANDRIS T., University of Minnesota
WILHELM, ROBERT C., Cornell University
WILLIS, JOHN S., Harvard University
WITTRY, SISTER ESPERANCE, College of St. Catherine
WOOD, LANGLEY H., Cornell University
YOW, FRANCIS W., Emory University

ECOLOGY

ABELES, FRED, Cornell University
BARBER, RICHARD I., Brown University
BARTH, ROBERT H., JR., Harvard University
BLUNT, SISTER MARION XAVIER, Marquette University
BOTHNER, RICHARD C., Fordham University
ELLSWORTH, JOANNE, Elmira College
GIFFORD, CAMERON E., Harvard University
RANDALL, DONALD, Oberlin College
STORY, LAWRENCE P., Drew University

3. FELLOWSHIPS AND SCHOLARSHIPS, 1957

Lucretia Crocker Scholarship:
GEORGE B. BOUCK, Botany Course

Conklin Scholarship:
ROBERT KIRCHEN, Embryology Course

Merkel Jacobs Scholarship:
MARGIT KAHLBROCK, Physiology Course

Calkins Scholarship:
THOMAS L. POULSON, Invertebrate Zoology Course

Bio Club Scholarships:
ARLEEN BRANNING, Invertebrate Zoology Course
IRWIN W. SHERMAN, Invertebrate Zoology Course

Linton Memorial Fund:
C. D. DIETER, Washington-Jefferson College

4. TABULAR VIEW OF ATTENDANCE, 1953–1957

	1953	*1954*	*1955*	*1956*	*1957*
INVESTIGATORS—TOTAL	310	298	250	304	326
Independent	176	180	162	184	186
Under Instruction	37	20	9	20	23
Library Readers	46	52	54	50	42
Research Assistants	51	46	25	50	75
STUDENTS—TOTAL	136	134	148	140	139
Zoology	55	56	56	55	55
Embryology	30	29	30	28	27
Physiology	31	28	30	30	30
Botany	11	12	19	18	18
Ecology	9	9	13	9	9
TOTAL ATTENDANCE	446	432	398	444	465
Less persons represented as both investigators and students		5		2	3
	446	427	398	442	462

INSTITUTIONS REPRESENTED—TOTAL	155	136	129	130	129
By investigators	90	104	95	97	94
By students	65	32	34	33	35
SCHOOLS AND ACADEMIES REPRESENTED					
By investigators		2	3	3	5
By students	1	1	2	1	1
FOREIGN INSTITUTIONS REPRESENTED					
By investigators	15	11	8	9	11
By students	6	13	6	6	5

5. INSTITUTIONS REPRESENTED, 1957

Amherst College
American Museum of Natural History
Boston University School of Medicine
Brooklyn College
Brown University
Bryn Mawr College
Chatham College
Children's Hospital of Philadelphia
City College of New York
Colby College
College of Mt. St. Joseph on the Ohio
Columbia University, College of Physicians and Surgeons
Columbia University, Zoology Dept.
Cornell University
Cornell University Medical School
Corporation of Roman Catholic Clergymen
Duke University
Albert Einstein College of Medicine
Elmira College
Emory University
Florida State University
Fordham University
Hahnemann Medical College
Harvard University
Harvard University Medical School
Indiana University
Institute for Muscle Research
Johns Hopkins University
Johns Hopkins University Medical School
Eli Lilly and Company
Marquette University
National Institutes of Health
New York University—Heights
New York University College of Medicine
New York University, Washington Square College
North Carolina State College
Northwestern University
Oberlin College
Princeton University
Purdue University
Radcliffe College
Rockefeller Institute for Medical Research
Rutgers University
Saint Joseph's College
St. Louis University
St. Louis University, School of Medicine
Single Cell Foundation
Sloan-Kettering Institute
Southwestern Medical College
State University of Iowa
State University of New York, College of Medicine at Syracuse
Syracuse University
Temple University
University of Chicago
University of Florida
University of Illinois
University of Illinois, College of Medicine
University of Maine
University of Michigan
University of Minnesota
University of New Hampshire
University of North Carolina
University of Oklahoma
University of Pennsylvania
University of Pennsylvania Medical School
University of Pittsburgh
University of Rochester
University of Vermont
University of Virginia, School of Medicine
University of Wisconsin
U. S. Fish and Wildlife Service
Vassar College
Veterans Administration Hospital of Brooklyn
Wabash College
Washington and Jefferson College
Washington University School of Medicine
Wellesley College
Wesleyan University
Wheaton College
Wilson College
Yale University

Supporting Institutions and Agencies, 1957

Abbott Laboratories
American Cancer Society
American Philosophical Society
Associates of the Marine Biological Laboratory
Atomic Energy Commission
Ciba Pharmaceutical Products, Inc.
The Grass Foundation
Kellogg Foundation
The Lalor Foundation
Eli Lilly and Company
Merck and Company, Inc.
National Institutes of Health
National Science Foundation
Office of Naval Research
The Rockefeller Foundation
Schering Corporation
Smith, Kline and French Foundation
The Upjohn Company

Foreign Institutions Represented, 1957

Zoologisches Institut, Mainz, Germany
McGill University, Montreal, Canada
Glasgow University, Scotland
University College, London, England
Wenner-Grens Institute, Sweden
University of Birmingham, England
University of Oslo, Sweden
University of Brussels, Belgium
Royal Veterinary College, Sweden
Utrecht University, The Netherlands
McMaster University, Hamilton College, Canada

6. Evening Lectures, 1957

July 5
Bentley Glass ..... "In pursuit of a gene"
July 12
K. Linderström-Lang ..... "Deuterium exchange of proteins in aqueous solution"
July 19
Olov Lindberg ..... "Functional-structural correlations in mitochondria"
July 26
Albert I. Lansing ..... "Chemical morphology of the elastic fiber"
August 2
James D. Ebert ..... "The acquisition of biological specificity"
August 9
J. C. Eccles ..... "The behavior of nerve cells"
August 16
Francis J. Ryan ..... "Mutation as an error in gene duplication"
August 23
Seymour S. Cohen ..... "The chemical pathology of the virus infected cell"

7. Tuesday Evening Seminars, 1957

July 2
Charles B. Metz ..... "The enhancement of starfish sperm motility and respiration by metals and metal binding agents"
Norman E. Kemp ..... "Differentiation of cortical cytoplasm and extra-cellular membranes of oocytes, including changes at fertilization"

LAURA HUNTER COLWIN and ARTHUR L. COLWIN ..... "Lytic and other activities of the individual spermatozoon during the early events of sperm entry (Hydroides, Saccoglossus, and several other invertebrates)"

July 9

A. M. SHANES ..... "Ion movement in vertebrate nerve"

WILLIAM STEPHENSON ..... "Relationships between ion movements and membrane potential changes in muscle"

G. HOYLE ..... "Nervous control of muscular contraction in arthropods"

W. H. FREYGANG, JR. ..... "Evidence for electrical inexcitability of neuron soma"

July 16

T. R. TOSTESON, S. A. FERGUSON and L. V. HEILBRUNN ..... "Further studies of the antimitotic and carcinostatic action of ovarian extracts"

L. V. HEILBRUNN, FRANCIS ASHTON, CARL FELDHERR and W. L. WILSON .. "The action of insulin on living cells"

FRANCIS ASHTON ..... "Magnetic studies on cells and protoplasm"

CARL FELDHERR ..... "The metachromatic reaction in various types of protoplasm"

PETER RIESER ..... "Effect of x-rays on fibrinogen"

PAUL R. GROSS, SYLVAN NASS and WILLIAM PEARL ..... "Mechanisms of sol-gel transformations in the cytoplasm"

July 23

R. E. BENESCH and R. BENESCH ..... "Sulfur linkages in hemoglobins"

A. CHASE ..... "Uricase inactivation by urea"

L. LORAND ..... "Clotting of blood: a study of the polymerization of proteins"

H. K. SCHACHMAN ..... "Structural considerations on bushy stunt virus"

July 30

LUIGI PROVASOLI ..... "Effect of plant hormones on sea weed"

DWIGHT MCNAIR SCOTT ..... "Changes in RNA during synchronous division of E. coli"

JAY S. ROTH ..... "Observations on the RNase system of rat liver"

BERNARD DAVIS ..... "Bacterial permease systems"

August 6

JOSEPH GALL ..... "Thymidine incorporation into the macronucleus of Euplotes (Protozoa)"

BEAL B. HYDE ..... "The effect of Versene on the sulfhydryls of chromatin"

C. W. METZ ..... "Interactions between chromosomes and cytoplasm during early embryonic development in Sciara (Diptera)"

August 13

Bostwick H. Ketchum ..... "Marine ecology and its place in biological research"

Eugene P. Odum ..... "Studies on simple natural ecosystems"

John H. Ryther ..... "On the efficiency of photosynthesis in the sea"

Thomas S. Austin ..... "The ecology of the biota of the equatorial Pacific"

August 20

L. Lorand, J. Molnar and C. Moos .... "Biochemical studies of relaxation in glycerinated muscle"

F. D. Carlson and A. Siger ..... "Creatine phosphate and adenosintriphosphate breakdown in iodoacetate poisoned muscle"

A. G. Szent-Györgyi and Carolyn Cohen ..... "Structural aspects of muscle proteins"

T. Hayashi, R. Strohman and R. Rosenbluth ..... "Myosin and actin interaction, and construction"

8. Members of the Corporation, 1957

1. Life Members

Brodie, Mr. Donald M., 522 Fifth Avenue, New York 18, New York
Calvert, Dr. Philip P., University of Pennsylvania, Philadelphia, Pennsylvania
Carver, Dr. Gail L., Mercer University, Macon, Georgia
Cole, Dr. Elbert C., 2 Chipman Park, Middlebury, Vermont
Cowdry, Dr. E. V., Washington University, St. Louis, Missouri
Crane, Mrs. W. Murray, Woods Hole, Massachusetts
Dederer, Dr. Pauline H., Connecticut College, New London, Connecticut
Dungay, Dr. Neil S., Carleton College, Northfield, Minnesota
Goldfarb, Dr. A. J., College of the City of New York, New York City, New York
Knowlton, Dr. F. P., 1356 Westmoreland Avenue, Syracuse, New York
Lewis, Dr. W. H., Johns Hopkins University, Baltimore, Maryland
Lowther, Dr. Florence deL., Barnard College, New York City, New York
MacNaught, Mr. Frank M., Woods Hole, Massachusetts
Malone, Dr. E. F., 6610 North 11th Street, Philadelphia 26, Pennsylvania
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Moore, Dr. J. Percy, University of Pennsylvania, Philadelphia, Pennsylvania
Payne, Dr. Fernandus, Indiana University, Bloomington, Indiana
Porter, Dr. H. C., University of Pennsylvania, Philadelphia, Pennsylvania
Riggs, Mr. Lawrason, 74 Trinity Place, New York 6, New York
Scott, Dr. Ernest L., Columbia University, New York City, New York
Turner, Dr. C. L., Northwestern University, Evanston, Illinois
Waite, Dr. F. G., 144 Locust Street, Dover, New Hampshire
Wallace, Dr. Louise B., 359 Lytton Avenue, Palo Alto, California
Warren, Dr. Herbert S., 610 Montgomery Avenue, Bryn Mawr, Pennsylvania
Young, Dr. B. P., Cornell University, Ithaca, New York

2. Regular Members

Abell, Dr. Richard G., 7 Cooper Road, New York City, New York
Adams, Dr. A. Elizabeth, Mount Holyoke College, South Hadley, Massachusetts
Addison, Dr. W. H. F., 286 East Sidney Avenue, Mount Vernon, New York
Adolph, Dr. Edward F., University of Rochester, School of Medicine and Dentistry, Rochester, New York
Albert, Dr. Alexander, Mayo Clinic, Rochester, Minnesota
Allen, Dr. M. Jean, Department of Biology, Wilson College, Chambersburg, Pennsylvania
Allen, Dr. Robert D., Department of Biology, Princeton University, Princeton, New Jersey
Alscher, Dr. Ruth, Department of Physiology, Manhattanville College, Purchase, New York
Amberson, Dr. William R., Department of Physiology, University of Maryland School of Medicine, Baltimore, Maryland
Anderson, Dr. J. M., Department of Zoology, Cornell University, Ithaca, New York
Anderson, Dr. Rubert S., Medical Laboratories, Army Chemical Center, Maryland (Box 632 Edgewood, Maryland)
Anderson, Dr. T. F., c/o Dr. A. Lurff, Institut Pasteur, 28 Rue du Dr. Roux, Paris 15e, France
Armstrong, Dr. Philip B., State University of New York College of Medicine, Syracuse 10, New York
Arnold, Dr. William A., Oak Ridge National Laboratory, Oak Ridge, Tennessee
Atwood, Dr. Kimball C., 68½ Outer Drive, Oak Ridge, Tennessee
Austin, Dr. Mary L., Wellesley College, Wellesley, Massachusetts
Ayers, Dr. John C., Department of Zoology, University of Michigan, Ann Arbor, Michigan
Baitsell, Dr. George A., Osborn Zoological Laboratories, Yale University, New Haven, Connecticut
Baker, Dr. H. B., Zoological Laboratory, University of Pennsylvania, Philadelphia, Pennsylvania
Ball, Dr. Eric G., Department of Biological Chemistry, Harvard University Medical School, Boston 15, Massachusetts
Bang, Dr. F. B., Department of Pathobiology, Johns Hopkins University School of Hygiene, Baltimore 5, Maryland
Ballard, Dr. William W., Dartmouth College, Hanover, New Hampshire
Bard, Dr. Philip, Johns Hopkins Medical School, Baltimore, Maryland
Barth, Dr. L. G., Department of Zoology, Columbia University, New York City, New York
Bartlett, Dr. James H., Department of Physics, University of Illinois, Urbana, Illinois
Beams, Dr. Harold W., Department of Zoology, State University of Iowa, Iowa City, Iowa
Beck, Dr. L. V., Department of Physiology and Pharmacology, University of Pittsburgh School of Medicine, Pittsburgh 13, Pennsylvania
Beers, Dr. C. D., University of North Carolina, Chapel Hill, North Carolina

BEHRE, DR. ELINOR H., Louisiana State University, Baton Rouge, Louisiana
BENESCH, DR. REINHOLD, Marine Biological Laboratory, Woods Hole, Massachusetts
BENESCH, DR. RUTH, Marine Biological Laboratory, Woods Hole, Massachusetts
BENNETT, DR. MIRIAM, Department of Biology, Sweet Briar College, Sweet Briar, Virginia
BERG, DR. WILLIAM E., Department of Zoology, University of California, Berkeley, California
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BERTHOLF, DR. FLOYD M., College of the Pacific, Stockton, California
BEVELANDER, DR. GERRIT, New York University School of Medicine, New York 16, New York
BIGELOW, DR. HENRY B., Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts
BISHOP, DR. DAVID W., Department of Embryology, Carnegie Institution of Washington, Baltimore 5, Maryland
BLANCHARD, DR. K. C., Johns Hopkins Medical School, Baltimore, Maryland
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BODIAN, DR. DAVID, Department of Epidemiology, Johns Hopkins University, Baltimore 5, Maryland
BOELL, DR. EDGAR J., Yale University, New Haven, Connecticut
BOETTIGER, DR. EDWARD G., Department of Zoology, University of Connecticut, Storrs, Connecticut
BOLD, DR. HAROLD C., Department of Botany, University of Texas, Austin, Texas
BOREI, DR. HANS, Department of Zoology, University of Pennsylvania, Philadelphia, Pennsylvania
BOWEN, DR. VAUGHAN T., Woods Hole Oceanographic Institution, Woods Hole, Massachusetts
BRADLEY, DR. HAROLD C., 2639 Durant Avenue, Berkeley 4, California
BRIDGMAN, DR. ANNA J., Department of Biology, Agnes Scott College, Decatur, Georgia
BRONK, DR. DETLEV W., Rockefeller Institute, 66th Street & York Avenue, New York 21, New York
BROOKS, DR. MATILDA M., Department of Physiology, University of California, Berkeley 4, California
BROWN, DR. DUGALD E. S., Department of Zoology, University of Michigan, Ann Arbor, Michigan
BROWN, DR. FRANK A., JR., Department of Biological Sciences, Northwestern University, Evanston, Illinois

BROWNELL, DR. KATHERINE A., Ohio State University, Columbus, Ohio
BUCK, DR. JOHN B., Laboratory of Physical Biology, National Institutes of Health, Bethesda, Maryland (10405 Muir Place, Kensington, Maryland)
BULLINGTON, DR. W. E., Randolph-Macon College, Ashland, Virginia
BULLOCK, DR. T. H., Department of Zoology, University of California, Los Angeles 24, California
BURBANCK, DR. WILLIAM D., Box 834, Emory University, Georgia
BURDICK, DR. C. LALOR, The Lalor Foundation, 4400 Lancaster Pike, Wilmington, Delaware
BURKENROAD, DR. M. D., c/o Lab. Nal. de Pesca, Apartado 3318, Estofeta #1, Olindania, Republic of Panama
BUTLER, DR. E. G., Department of Biology, Princeton University, Princeton, New Jersey
CAMERON, DR. J. A., Baylor College of Dentistry, Dallas, Texas
CANTONI, DR. GIULIO, National Institutes of Health, Mental Health, Bethesda 14, Maryland
CARLSON, DR. FRANCIS D., Department of Biophysics, Johns Hopkins University, Baltimore, Md.
CARPENTER, DR. RUSSELL L., Tufts College, Medford 55, Massachusetts
CARSON, MISS RACHEL, 204 Williamsburg Drive, Silver Spring, Maryland
CATTELL, DR. MCKEEN, Cornell University Medical College, 1300 York Avenue, New York City, New York
CATTELL, MR. WARE, Cosmos Club, Washington 5, D. C.
CHAET, DR. ALFRED B., Boston University School of Medicine, 80 E. Concord Street, Boston 18, Massachusetts
CHAMBERS, DR. EDWARD, Department of Physiology, University of Miami Medical School, Coral Gables, Florida
CHANG, DR. JOSEPH J., National Institute of Neurological Diseases and Blindness, National Institutes of Health, Bethesda, Maryland
CHASE, DR. AURIN M., Department of Biology, Princeton University, Princeton, New Jersey
CHENEY, DR. RALPH H., Biology Department, Brooklyn College, Brooklyn 10, New York
CLAFF, MR. C. LLOYD, 5 Van Beal Road, Randolph, Massachusetts
CLARK, DR. A. M., Department of Biological Sciences, University of Delaware, Newark, Delaware
CLARK, DR. E. R., The Wistar Institute, Woodland Avenue and 36th Street, Philadelphia 4, Pennsylvania
CLARK, DR. LEONARD B., Department of Biology, Union College, Schenectady, New York
CLARKE, DR. GEORGE L., Harvard University, Biological Laboratory, Cambridge 38, Massachusetts
CLELAND, DR. RALPH E., Indiana University, Bloomington, Indiana
CLEMENT, DR. A. C., Department of Biology, Emory University, Emory, Georgia
CLOWES, DR. G. H. A., Eli Lilly and Company, Indianapolis, Indiana
COE, DR. W. R., 183 Third Avenue, Chula Vista, California
COHEN, DR. SEYMOUR S., Department of Physiological Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania

COLE, DR. KENNETH S., National Institutes of Health (NINDB), Bethesda 14, Maryland
COLLETT, DR. MARY E., 34 Weston Road, Wellesley 81, Massachusetts
COLLIER, DR. JACK R., Department of Zoology, Louisiana State University, Baton Rouge, Louisiana
COLTON, DR. H. S., Box 601, Flagstaff, Arizona
COLWIN, DR. ARTHUR L., Department of Biology, Queens College, Flushing, New York
COLWIN, DR. LAURA H., Department of Biology, Queens College, Flushing, New York
COOPERSTEIN, DR. SHERWIN J., Department of Anatomy, Western Reserve University Medical School, Cleveland, Ohio
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COPELAND, DR. MANTON, Bowdoin College, Brunswick, Maine
COPLEY, DR. ALFRED L., Centre National de Transfusion Sanguine, 6, Rue Alexandra-Cobonel, Paris XVe, France
CORNMAN, DR. IVOR, Hazleton Laboratories, Box 333, Falls Church, Virginia
COSTELLO, DR. DONALD P., Department of Zoology, University of North Carolina, Chapel Hill, North Carolina
COSTELLO, DR. HELEN MILLER, Department of Zoology, University of North Carolina, Chapel Hill, North Carolina
CRANE, MR. JOHN O., Woods Hole, Massachusetts
CROASDALE, DR. HANNAH T., Dartmouth College, Hanover, New Hampshire
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Keith, Mr. and Mrs. Harold C.
King, Mr. Franklin
Koller, Mrs. Lewis
Lemann, Mrs. Solen B.
Lobb, Mrs. John
Lurdon, Mr. W. R.
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V. REPORT OF THE LIBRARIAN

In 1957, seventy-six new journals were acquired, bringing the total number of currently received titles to 1635. Of these titles, there were 490 (15 new) Marine Biological Laboratory subscriptions; 617 (14 new) exchanges and 192 (21 new) gifts; 90 (9 new) were Woods Hole Oceanographic Institution subscriptions; 191 (7 new) were exchanges and 55 (10 new) were gifts. During the past ten years, we averaged 60 new journals per year. The ever growing number of new journals being issued far exceeds the number which cease publication.

The Laboratory purchased 151 books, received 61 complimentary copies (4 from authors and 57 from publishers), and accepted 13 miscellaneous gifts. The Institution purchased 39 titles and received 10 gifts. The total number of books accessioned amounted to 274.

By purchase and by gift the Laboratory completed 13 journal sets and partially completed 19. The Institution completed 4 sets and partially completed 3. There were 3920 reprints added to the collection, of which 2055 were of current issue.

At the close of the year, the Library contained 67,961 bound volumes and 206,125 reprints.

The Library sent out on inter-library loan 243 volumes and borrowed 115 for the convenience of the scientists. It is hoped that a copying machine may be purchased in the near future so that short papers may be reproduced for out-of-town loans, thus eliminating some of the depreciation on our volumes. A process such as this could also be utilized for summer service.

Reprint collections were received from the estate of Dr. Arthur Weysse and from the University of Pittsburgh; many books, journal numbers and papers were received from Drs. Ethel B. Harvey, C. Ladd Prosser, Rufus R. Humphrey, Phineas W. Whiting, Paul S. Galtsoff, Ralph Wichterman, and the Tompkins-McCaw Library, Medical College of Virginia. Dr. Alfred W. Senft kindly donated back volumes and a current subscription to the "New England Journal of Medicine." Grateful acknowledgment is herewith extended to the donors of these very acceptable presentations.

With a larger sum available for the purchase of books, and with the many suggestions so willingly submitted by the Library Advisory Committee, we were in a position in 1957 to add many new titles to the shelves. An increase in the binding budget also enabled us to have bound 275 back volumes, bringing the total to 1110 for the year. This same degree of progress is anticipated in 1958.

Respectfully submitted,

DEBORAH L. HARLOW,
Librarian

VI. REPORT OF THE TREASURER

The market value of both the General Fund and the Library at December 31, 1957, amounted to $1,461,278 as compared with the total of $1,472,265 as of December 31, 1956. The average yield on the securities was 3.84% of market value and 5.60% of book value. The total uninvested principal cash in the above accounts as of December 31, 1957, was $2,248. Classification of the securities held in the Endowment Funds appears in the auditor's report.

The market value of the pooled securities as of December 31, 1957, was $247,629 with uninvested principal cash of $102. The book value of the securities in this account was $236,735. The average yield on market value was 3.88% and 4.06% of book value.

The proportionate interest in the Pooled Fund account of the various Funds as of December 31, 1957, is as follows:

Pension Fund	17.608%
General Laboratory Investment	57.866
Other:	
Bio Club Scholarship Fund	1.687
Rev. Arsenious Boyer Scholarship Fund	2.064
Gary N. Calkins Fund	1.933
Allen R. Memhard Fund	.374
F. R. Lillie Memorial Fund	6.515

Lucretia Crocker Fund	7.054
E. G. Conklin Fund	1.194
M. H. Jacobs Scholarship Fund	.850
Jewett Memorial Fund	.626
Anonymous Gift	2.229

The Pooled Fund includes the Jewett Memorial Fund and an anonymous Gift Fund which were additions during 1957. The Jewett Memorial Fund was created by gifts in memory of the late George Frederick Jewett. Mr. Jewett as well as his father and mother and the other members of his family have been keenly interested in the Laboratory since its inception. It has not yet been determined how the Jewett Fund and the fund created by the anonymous gift will be used, but the views of the Jewett family and the donor of the latter fund will be given first consideration.

Considerable activity was recorded in the special custodian account owing to the purchase of short-term Government bonds to activate available cash which would otherwise remain idle in our regular cash accounts pending payment of construction expenses. Income earned was $646.40.

Inasmuch as the MBL Club loan was reduced to $2,052, the securities pledge to cover this loan was reduced to $3,000.

Donations from MBL Associates for 1957 were $3,481 as compared with $5,255 in 1956. Unrestricted gifts from foundations, societies and companies amounted to $33,000.

For the rehabilitation of the Crane Building, the National Science Foundation advanced $415,000 in 1957. Construction began in September and is scheduled for completion in May of 1958.

In April of 1957 we paid off the David House Mortgage in the amount of $5,000.

Lynbrand, Ross Bros. & Montgomery have examined our books and submitted financial statements for examination.

Following is a statement of the auditors.

To the Trustees of the Marine Biological Laboratory, Woods Hole, Massachusetts:

We have examined the balance sheets of Marine Biological Laboratory as at December 31, 1957, the related statements of operating expenditures and income for the year then ended, and statement of current fund for the year ended December 31, 1957. Our examination was made in accordance with generally accepted auditing standards, and accordingly included such tests of the accounting records and such other auditing procedures as we considered necessary in the circumstances.

In our opinion, the accompanying financial statements present fairly the assets, liabilities and funds of Marine Biological Laboratory at December 31, 1957, and the expenditures and income for the year then ended.

LYBRAND, ROSS BROS. & MONTGOMERY

Boston, Massachusetts
May 22, 1958

JAMES H. WICKERSHAM,
Treasurer

MARINE BIOLOGICAL LABORATORY

Balance Sheet

December 31, 1957

Investments

Investments held by Trustee:	
Securities, at cost (approximate market quotation $1,461,278)	$1,002,682
Cash	2,248
	1,004,930
Investments of other endowment and unrestricted funds:	
Pooled Investments, at cost (approximate market quotation $247,629)	236,735
Less temporary investment of current fund cash	5,728
	231,007
Other investments (Note A)	67,323
Cash	11,263
Accounts receivable	5,038
	314,631

Plant Assets

Land, buildings, library and equipment (Note B)	2,517,845
Less allowance for depreciation (Note B)	1,026,681
	1,491,164
Construction in progress	103,856
Cash	34,560
U. S. Treasury bills, due 1/30/58, at cost (face value $350,000)	346,815
	1,976,395

Current Assets

Cash	142,160
U. S. Treasury bills, at cost:	
$40,000 face value due 2/13/58	39,649
Temporary investment in pooled securities	5,728
Accounts receivable (U. S. Government $19,605)	36,274
Inventories of specimens and Bulletins	57,282
Prepaid insurance and other	13,531
	$3,590,580

Notes:

A—The Laboratory has guaranteed a note of approximately $2,400 of the M.B.L. Club and has pledged as security therefor bonds with an original cost of $3,000 included in other investments.

B—The Laboratory has since January 1, 1916, provided for reduction of book amounts of plant assets and funds invested in plant at annual rates ranging from 1% to 5% of the original cost of the assets.

MARINE BIOLOGICAL LABORATORY

Balance Sheet

December 31, 1957

Endowment Funds

Endowment funds given in trust for benefit of the Marine Biological Laboratory ..		$1,004,930
Endowment funds for awards and scholarships:		
Principal	$ 64,415	
Unexpended income	2,428	66,843
Unrestricted funds functioning as endowment		206,378
Retirement fund		46,233
Pooled investments—accumulated gain or (loss)		(4,823)
		314,631

Plant Liability and Funds

Funds expended for plant, less retirements	$2,551,469	
Less allowance for depreciation charged thereto	1,026,681	1,524,788
Unexpended plant funds		381,375
		1,906,163
Accounts payable		70,232
		1,976,395

Current Liabilities and Funds

Accounts payable	43,409
Unexpended balances of gifts for designated purposes	8,744
Advance payments on research contracts	94,217
Current fund	148,254
	$3,590,580

MARINE BIOLOGICAL LABORATORY

Statement of Operating Expenditures and Income

Year Ended December 31, 1957

Operating Expenditures

Direct expenditures of departments:	
Research and accessory services	$146,859
Instruction	35,237
Library, including book purchases	32,712
Biological Bulletin	16,995
	231,803
Direct costs on research contracts	129,983
Administration and general	54,526
Plant operation and maintenance	81,156
Dormitories and dining services	143,322
Plant additions from current funds	59,581
	700,371
Less depreciation included in plant operation and dormitories and dining services above but charged to plant funds	36,351
	664,020

Income

Direct income of departments:	
Research fees	43,418
Accessory services (including sales of biological specimens $67,562)	103,718
Instruction fees	16,980
Library fees and income	8,239
Biological Bulletin, subscriptions and sales	19,846
	192,201
Reimbursement and allowance for direct and indirect costs on research contracts	151,444
Dormitories and dining services income	108,349
	451,994
Investment income used for current expenses:	
Endowment funds	83,984
Current fund investments	1,645
Gifts used for current expenses	127,301
Sundry income	175
Total current income	665,099
Excess of income	1,079

MARINE BIOLOGICAL LABORATORY

Statement of Current Fund

Year Ended December 31, 1957

Balance January 1, 1957	$147,175
Excess of income over operating expenditures 1957	1,079
Balance December 31, 1957	$148,254

MARINE BIOLOGICAL LABORATORY

Summary of Investments

December 31, 1957

	Cost	% of Total	Approximate Market Quotations	% of Total	Investment Income 1957
Securities held by Trustee:					
General endowment fund:					
U. S. Government bonds	$ 81,000	9.7	$ 81,000	6.8	$ 2,359
Other bonds	420,980	50.2	403,589	33.7	12,246
	501,980	59.9	484,589	40.5	14,605
Preferred stocks	85,788	10.2	71,713	6.0	3,370
Common stocks	251,097	29.9	641,355	53.5	28,312
	838,865	100.0	1,197,657	100.0	46,287
General Educational Board endowment fund:					
U. S. Government bonds	25,000	15.3	25,000	9.5	749
Other bonds	70,530	43.0	68,813	26.1	2,327
	95,530	58.3	93,813	35.6	3,076
Preferred stocks	27,281	16.7	24,337	9.2	1,130
Common stocks	41,006	25.0	145,471	55.2	5,608
	163,817	100.0	263,621	100.0	9,814
Total securities held by Trustee	$1,002,682		$1,461,278		$56,101
Investments of other endowment and unrestricted funds:					
Pooled investments:					
U. S. Government bonds	—	—	—	—	833
Other bonds	138,302	58.4	141,416	57.1	3,105
	138,302	58.4	141,416	57.1	3,938
Common stocks	98,433	41.6	106,213	42.9	5,676
	236,735	100.0	$ 247,629	100.0	9,614
Other investments:					
U. S. Government bonds	2,970				131
Common stocks	43,600				23,444
Real estate and mortgage	20,753				—
	67,323				23,575
Total investments of other endowment and unrestricted funds	$ 304,058				$33,189
Total investment income					89,290
Custodian's fee charged thereto					(574)
Income of current funds temporarily invested in pooled securities					(204)
Investment income distributed to funds					$88,512

COELOMIC CORPUSCLES OF ECHINODERMS [1]

RICHARD A. BOOLOOTIAN [2] AND ARTHUR C. GIESE

Hopkins Marine Station of Stanford University, Pacific Grove, California

Although a variety of corpuscles have been described during the last century by investigators of echinoderm perivisceral fluid, disagreement exists among the descriptions of different authors and a re-investigation of the problem with newer methods is desirable before the corpuscles of echinoderm perivisceral fluid can be properly characterized. These newer methods are primarily observation through the phase contrast microscope, so effective in Grégoire's studies (1953) on insect blood, and observation of cells unaltered by contact with air, glass or chemicals which Hensill (1949) found so useful in his study of crab blood. In addition, the study of all the transformations of a cell of a given type under gradually altered conditions discloses changes from one cell type to another in some instances. Furthermore, a comparative study made possible a useful tentative classification of the cells found in fifteen species of echinoderms representing all the living classes of Echinodermata.

Materials and Methods

The animals were collected in the vicinity of the Monterey Peninsula at low tide in some cases and by dredging in others. The animals were used as soon after collection as possible since starvation is known to alter clotting (Glavind, 1948). Cell types of each species were determined by the examination of fluid drawn from the perivisceral cavity with the aid of a siliconized syringe. A drop of the fluid was placed on a siliconized cover slip which was inverted over a depression slide, and examined immediately at magnifications of 43 × and 97 × and photographed periodically.

The optical equipment consisted of a Spencer 18 ML phase microscope equipped with a Spencer phase turret condenser, bright contrast objectives and wide field oculars. The source of illumination was an Ortho-Illuminator-B (American Optical Co.), using 100–300 watt bulbs.

The photomicrographic equipment used was a Kine-Exacta model VX camera coupled to a Leitz Micro-Ibso attachment. Exposures were made on Microfile film which was developed in D-11 developer and printed on single weight glossy surface DuPont Varigram paper.

Since contact with air is known to alter the morphology of cells, the perivisceral fluid was taken up into evacuated capillaries. The capillaries were prepared by

[1] Supported in part by National Science Foundation Grant GS-482 and Public Health Grant RG-4578 (C).

We are also indebted to Dr. A. R. Moore for his sustained interest and suggestions, to Dr. L. Blinks for accommodations and suggestions, to Dr. R. L. Bolin for extending use of facilities and for helpful criticism, and to Mr. A. Farmanfarmaian for counsel and advice.

[2] Now at the Department of Zoology, University of California at Los Angeles.

pulling 5-mm. Pyrex tubing in such a manner that the capillary diameters never exceeded 1 mm.

The inner walls of the capillaries were coated with silicon (G.E. Dri-Film) by aspirating the reagent and subsequent drying. They were then flame-sealed at one end, evacuated, and flame-sealed at the other end in 7.5-cm. segments. Silicon was used because it coats the glass and prevents cytolysis of cells coming in contact with clean glass (Jacques *et al.*, 1946). Each capillary was scratched with a carborundum point half a centimeter from one end. The scratched end inserted through the peristome (echinoids) or a dermal branchia (asteroid) can be broken at the scratch by a slight pressure, and the body fluid is aspirated into the capillary. In the case of holothuroids a longer capillary, scratched in the center, was inserted into the interambulacral margin of the animal and broken in the middle in the same manner. The open tip of the capillary was covered with silicon grease upon removal. Then the capillary was placed on a slide in a channel filled with glycerine and covered with a cover slip. With this method it is possible to study types for at least five minutes before clotting appears, and to observe any changes which occur during this time. Furthermore, the capillary tubes can be rotated and the nature of the corpuscles ascertained in three dimensions. Clots also can be studied effectively in such preparations. This method readily lends itself to photography.

In order to determine which coelomic cells, if any, were phagocytic, one ml. of finely ground carmine suspension in sea water was injected by a syringe through the peristomial membrane in echinoids, through a dermal branchia in asteroids and through the body wall in holothuroids. At various time intervals, ranging from ten minutes to five days, hanging drop and capillary-tube preparations of the perivisceral fluid were examined and photographed.

Classification of Corpuscles of Echinoderm Body Fluids

The results of the present study, documented in succeeding sections, revealed thirteen types of fairly distinct cells (see Tables I and II). Some of these corpuscles appear to be phyletic in distribution, *e.g.*, the bladder amebocytes (Fig. 1) and the filiform amebocytes (Fig. 2), the first of which occur in thirteen of the species examined and the latter in twelve of the species examined. As will be discussed later, these two cell types are different phases of the same cell, e.g., in *Pisaster ochraceus*. The small spherical amebocytes (Fig. 3) are found in three of the asteroids investigated and in the ophiuroid and the crinoid. The fusiform corpuscle (Fig. 4), the vibratile corpuscle (Fig. 6), the eleocyte (Fig. 7), and hyaline hemocyte (Fig. 8) are found in the sea urchins only. The colorless spherical amebocyte (Fig. 5) is common both to the sea urchins and sand dollars. The other types of cells have a rather limited distribution. The large spherical corpuscle (Fig. 9) and the red corpuscle (Fig. 10) are found in the sand dollar and the crinoid. The lobular corpuscle (Fig. 11), on the other hand, is limited to the crinoid only. The hyaline plasma amebocyte (Fig. 12) is found in the starfish *Poraniopsis*. Cells "staining" with osmic acid (Fig. 13) are observed only in the sand dollar.

Corpuscles of Asteroids

The fluid within the spacious coelomic cavity of the asteroids contains coelomocytes of fewer types than occur in other classes. Two main types of cells have been

TABLE I

Distribution of corpuscular elements

Species	Bladder amebocyte	Filiform amebocyte	Small spherical corpuscle	Fusiform corpuscle	Colorless spherule amebocyte	Vibratile corpuscle	Eleocyte amebocyte	Hyaline hemocyte	Large spherical corpuscle	Red corpuscle	Lobular corpuscle	Hyaline plasma amebocyte	Cells "*staining*" with osmic acid
Astropecten californicus	x	x											
Mediaster aequalis	x	x	x										
Poranioposis inflata	x	x	x									x	
Patiria miniata	x	x											
Pycnopodia helianthoides	x	x	x										
Pisaster ochraceus	x	x											
Pisaster giganteus	x	x											
Pisaster brevispinus	x	x											
Strongylocentrotus purpuratus	x	x		x	x	x	x	x					
Strongylocentrotus franciscanus	x	x		x	x	x	x	x					
Strongylocentrotus fragilis	x	x		x	x	x	x	x					
Dendraster excentricus	x				x				x	x			x
Gorgonocephalus eucnemis			x	x									
Heliometra glacialis			x	x					x	x	x		
Stichopus californicus	x	x			x	x							
Figure number	1	2	3	4	5	6	7	8	9	10	11	12	13

TABLE II

Properties of coelomic corpuscles

Cell type	Range or size in μ*	Color	Granules	Vacuoles	Function	Citation
Bladder amebocyte	9–51	colorless, gray	numerous black	several	phagocytic	Kindred, 1921
Filiform amebocyte	8–55	gray	several black	two–many	clot, phagocytic	Kindred, 1921
Small spherical amebocyte	4–8, 7–35	green, yellow, red	black and red	occasional	clot	Cuénot, 1888
Fusiform corpuscle	2–12 ×6–30	gray	0	0	?	Cuénot, 1891
Colorless spherical amebocyte	8–12 × 13.6–28	pale yellow	lobular	0	lipid transport?	Kindred, 1921
Vibratile corpuscle	3–11.7 × 9–44	gray	numerous black	numerous small	circulation?	Kindred, 1921
Eleocyte	11.2–29 ×6.8–8	red	small red	0	O_2 transport?	MacMunn, 1885
Hyaline hemocyte	9.2–13	pale yellow	0	numerous	clot	This paper
Large spherical corpuscle	19–25.6	slightly brownish	brown	several large	?	Bookhout *et al.*, 1940
Red corpuscle	5.5–11.2 × 8–16	red	0	0	?	This paper
Lobular corpuscle	16–27 × 19–28.8	gray	0	0	?	Cuénot, 1891
Hyaline plasma amebocyte	17.04–31.2	gray	black	several	?	This paper
Osmophilic cells	10–12.3	pale yellow	0	0	?	This paper

* Length or length and width. A wide range is observed in some cases because the same type of corpuscle is of a different size in different species in which it is found.

described by Théel (1919), Kindred (1924), Lison (1930), Goodrich (1919), Durham (1888), Geddes (1879, 1880), Cuénot (1891): amebocytes with ordinary slender pseudopodia (filiform amebocytes) and amebocytes with petaloid or bladder extrusions called pseudopodia (bladder amebocytes).

In the present study both bladder amebocytes (Fig. 1) and filiform amebocytes (Fig. 2) were found to be abundant in all species of asteroids investigated. Another cell type, a small spherical corpuscle with pigmented granules, however, was also found in *Pycnopodia helianthoides*, *Mediaster aequalis*, and *Poraniopsis inflata*. The cells of the first two species contain a light red or orange intracellular pigment which is particularly obvious when the cells are concentrated by centrifugation. In *Poraniopsis inflata* the pigmented corpuscles contain black granules, but not in conspicuous quantity.

CORPUSCLES OF ECHINOIDS

The body fluid of the echinoids contains several types of cells which have been studied most extensively by Geddes (1879), Cuénot (1891), Théel (1896), Kindred (1921), Behre (1932), Boliek (1935), Kuhl (1937), Bookhout and Greenburg (1940), Liebman (1950), and Schinke (1950). Six types of cells have been described by various workers: amebocytes with spiked pseudopodia, amebocytes with petaloid pseudopodia, colorless spherule amebocytes, greenish and yellowish

spherule amebocytes, red spherule amebocytes, and vibratile corpuscles which are small spherical cells provided with a flagellum.

Seven types of cells were identified in the body fluid of the echinoids investigated here: bladder amebocytes, filiform amebocytes, fusiform corpuscles, colorless

PLATE I

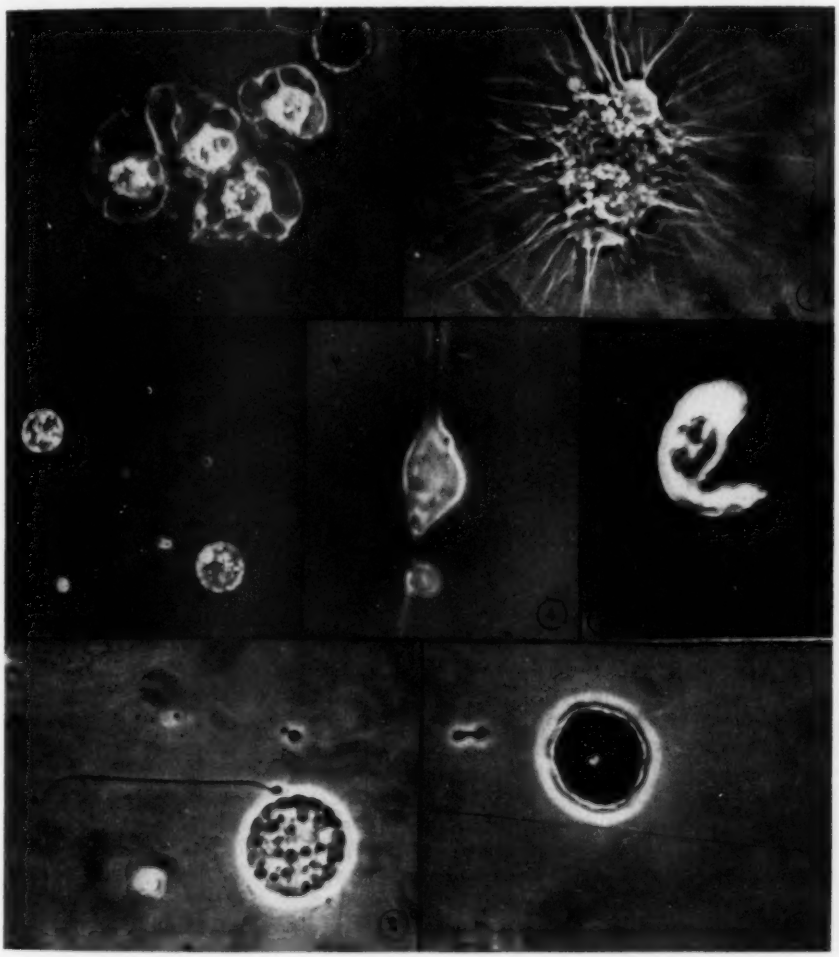

FIGURE 1, bladder amebocytes of *Dendraster excentricus*. FIGURE 2, filiform amebocyte of *Pisaster ochraceus*. FIGURE 3, small spherical corpuscle of *Heliometra glacialis*. FIGURE 4, fusiform corpuscle of *Strongylocentrotus franciscanus*. FIGURE 5, colorless spherule amebocyte of *Strongylocentrotus purpuratus*. FIGURE 6, vibratile corpuscle of *Strongylocentrotus purpuratus*. FIGURE 7, eleocyte of *Strongylocentrotus franciscanus*.

Photomicrographs were taken at a magnification of 344 × and were enlarged subsequently.

PLATE II

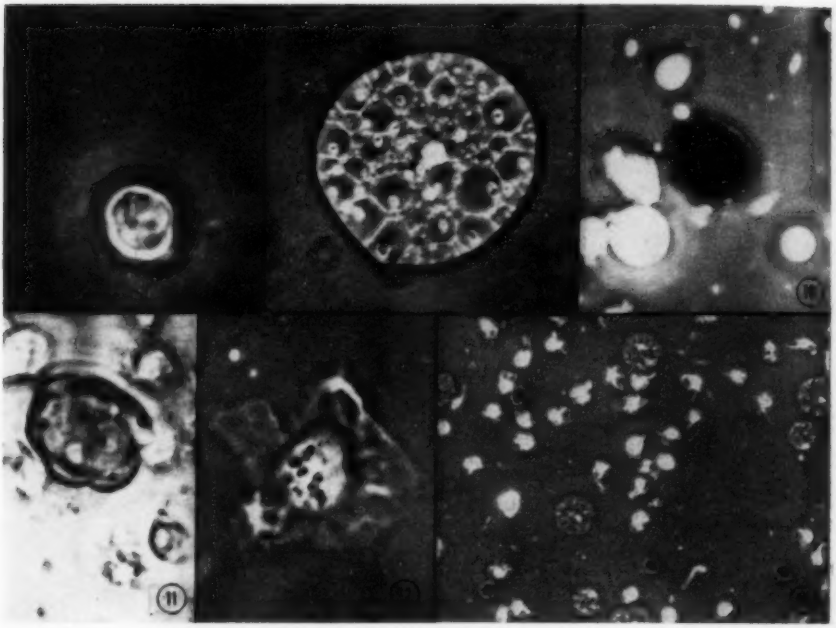

FIGURE 8, hyaline hemocyte of *Strongylocentrotus purpuratus.* FIGURE 9, large spherical corpuscle of *Dendraster excentricus.* FIGURE 10, red corpuscle of *Heliometra glacialis.* FIGURE 11, lobular corpuscle of *Heliometra glacialis.* FIGURE 12, hyaline plasma amebocyte of *Poraniopsis inflata.* FIGURE 13, osmophilic cells of *Dendraster excentricus* (small dark oval units).

Photomicrographs were taken at a magnification of 344 × and were enlarged subsequently.

spherule amebocytes, vibratile corpuscles, eleocytes, and hyaline hemocytes (Figs. 1, 2, 4, 5, 6, 7, 8).

The bladder amebocytes with large ectoplasmic extrusions and the filiform amebocytes are phagocytic. The eleocytes contain red spherules of echinochrome (MacMunn, 1885; Kuhn and Wallenfels, 1939), a common echinoid pigment which has been demonstrated in the three species of *Strongylocentrotus.* The colorless spherule amebocytes with spherical inclusions lack phagocytic power and move by extending broad round eruptive (guttata) pseudopodia. The fusiform corpuscles, which are few in number, have no known function. The hyaline hemocyte gives rise to an extracellular clot after disintegrating at the site of an injury. These disintegrating cells resemble the colorless spherule amebocytes to a considerable degree and, as a result, may have escaped the observations of past investigators because most of their observations were made on fixed and stained material.

The eleocytes have no known function, although several have been suggested by various investigators. Griffiths (1892) concluded that they are associated with oxygen transport as did Awerinzew (1911). Cuénot (1891) opposed this assump-

tion on the basis that no color change was observed when pigment was allowed to stand in air, and proposed that instead of being an oxygen-carrying agent, the eleocyte was a store of food material which the cells had taken from the intestine. The concentration of eleocytes in the body fluids differs in the three species of *Strongylocentrotus* studied. *Strongylocentrotus purpuratus* contains 1100 to 1700, *S. franciscanus*, 500 to 700, and *S. fragilis*, 100 to 150 cells/mm^3. The red cells are found in the epithelial lining of the sea urchin test and contribute to its color.

The vibratile corpuscles (Fig. 6) found in the sea urchins are of uncertain origin and function. They do not participate in clotting, except as they are incidentally caught in the mesh of fibers in the extensive clot formed in sea urchin body fluid. It is possible that the vibratile corpuscles aid in the mixing of the body fluid since the current created peripherally by the ciliated epithelium cannot extend far into the body mass. These cells were concentrated by fractional centrifugation (as were other types of corpuscles) and were kept alive in the body fluid of the urchin, *in vitro*, for many hours, but they were not observed to divide.

Corpuscles of Ophiuroids

The coelomic corpuscles of the ophiuroids have been least studied. Cuénot (1888) observed granular ameboid corpuscles with short spiked pseudopodia in an ophiuroid, and Kindred (1924) observed four types of cells: active leucocytes, passive leucocytes, colorless spherule amebocytes, and vibratile corpuscles in *Ophiopholis aculeata*. In the present study two cell types were observed in *Gorgonocephalus*—small spherical corpuscles (Fig. 3) and fusiform corpuscles (Fig. 4). The latter type of cell was not observed by Cuénot, while the small spherical corpuscle resembles that found by Cuénot. The function of these cells is unknown, although Cuénot has remarked that the ameboid granular cells (small spherical corpuscles) unite by their short pseudopodial tips and anastomose into a network, but he did not consider this to be clot formation.

Corpuscles of Crinoids

The coelomocytes of the crinoids have been described by Cuénot (1891), Hamann (1889), and Reichensperger (1912). Cuénot (1891) observed three kinds of cells, a small finely granular type with short spiked pseudopods, a larger pyriform or fusiform slow-moving cell filled with coarse spherules, and a cell filled with safranophil rods, which, according to Cuénot, was rounded when free in the coelomic fluid, pyriform when migrating through the tissues. Hamann observed numerous wandering ameboid cells in various crinoids and described two types, neither of which resembles Cuénot's forms. Reichensperger also noticed two kinds of coelomocytes in *Antedon:* a phagocytic ameboid form with short spiked pseudopods apparently identical with Cuénot's finely granular type and an oblong cell filled with numerous rods and granules.

In the species studied here, *Heliometra glacialis*, five types of cells were found (Table I), two of which are probably identical with the cells described by Cuénot, Hamann, and Reichensperger. The perivisceral fluid contained an abundance of ameboid cells with short pseudopodia (small spherical corpuscles, Figure 3). The second cell type (fusiform corpuscle, Figure 4), termed "pyriform" by Cuénot, was

not pear-shaped in *H. glacialis,* but more spindle-like. Occasional oblong bodies which approached pear-form were observed. The large spherical corpuscles, which lacked pseudopodial extensions, were abundant in the body fluid. The red corpuscles were found in small numbers and have not been reported by other investigators. The lobular corpuscles (Fig. 11), few in number, resemble an embryonic morula stage. The functions of these cells were not determined since the volume of fluid from the few specimens available was too small for extensive studies.

Corpuscles of Holothuroids

The coelomic cells of holothuroids have been described by numerous workers, particularly by Hérouard (1889), Becher (1907), Théel (1921), Kindred (1924), Ohuye (1934, 1936a, 1936b) and Endean (1958). They have been collectively called coelomocytes (Hyman, 1955). The cell types present vary in form, number, and size in different holothuroids, but the following kinds are common throughout the class: hemocytes, phagocytes, colorless spherule amebocytes and filiform amebocytes.

Hemocytes which have been reported for many species contain the pigment hemoglobin, as shown by Howell (1885, 1886), Van der Hyde (1922), Hogben and Van der Lingen (1928), and Kobayashi (1932), on *Thyone, Cucumaria, Paracaudina chilensis,* and *Molpadia roretzii,* respectively. They are not found in *Stichopus californicus,* the species studied here.

Phagocytes are found in all species so far studied. Various names such as cells with elongated pseudopodia (Hérouard), hyaline ameboid corpuscles (Ohuye), and bladder amebocytes (Kindred, 1924) have been applied to them. The term, bladder amebocyte (Fig. 1), is preferable since the large bladder-like projections are readily observable when viewed three-dimensionally.

The colorless spherule amebocytes (Fig. 5) were abundant in *Stichopus californicus.* Hamann (1883) designated these as plasma wandering cells. Cuénot (1891), who identified them as muriform cells, considered the proteinaceous spherules to be food reserves.

The homogeneous amebocytes, which lack inclusions, have been reported by Hamann (1883) and Becher (1907). This type of cell is rare and Hyman (1955) considers it a developmental stage of other cell types. It was not found in *Stichopus.*

Théel, Kawamoto, and Ohuye observed crystal-containing cells in several species of holothuroids. The crystals are in the cytoplasm and are mostly rhomboidal in shape. No crystal-containing cells were observed in *Stichopus.*

A cell type which has not been previously reported by investigators in holothuroids is the filiform amebocyte (Fig. 2). In *Stichopus californicus* these cells are actively involved in clot formation and also exhibit phagocytosis.

Discussion

Many types of coelomic corpuscles have been described by various investigators of echinoderm body fluids, most of whom fixed and stained the cells or used live cells without preventing degenerative changes following contact with glass or air. As a consequence their results were not entirely convincing. In the present study in which pains were taken to avoid the above pitfalls, many of the same cell types

were seen. However, more confidence may now be attached to the cell types described by the earlier workers, since their appearance has been checked with live cells under conditions which at least delay changes in cells occurring with clotting or agglutination.

Such coelomic cells as were not seen in the preparation made here, but which have been described by previous workers, may constitute additional cell types since the species used in the present study were not the same as theirs. Only future work using the same species of organism, can resolve this uncertainty. In the special case of the hemocytes—hemoglobin-containing cells of certain holothuroids—no question exists of their reality, even though they were not observed in the species of holothuroid used here (*Stichopus californicus*), since hemocytes have been observed in live specimens and recorded many times by various authors.

Some types of coelomocytes were observed in the species examined here which had not been previously described, *e.g.* the red corpuscles of the sand dollar and the crinoid, and the lobular corpuscles of the crinoid.

The existence of bladder amebocytes need no longer be questioned, even though the bladders appear to be petaloid rather than vesicular in fixed preparations (Goodrich, 1919). Examined in three dimensions, the bladder-like nature of the ectoplasmic extrusions is readily observable.

It was possible to resolve one controversy which occurs in the literature concerning the possible identity of the bladder amebocytes and the filiform amebocytes in asteroids. Théel (1919) and Kindred (1924) state these are merely phases of one another but cite no convincing evidence, and others question this conclusion. In observations on body fluids of several asteroids, the fresh sample showed a predominance of bladder amebocytes, but upon standing, the same preparation shows a predominance of filiform amebocytes. If the filiform amebocytes represent a pre-coagulation change, it should be possible to prevent this with an anticoagulant such as cysteine. Cysteine-treated coelomic fluid was found to contain only bladder amebocytes when examined at various time intervals in *Pisaster ochraceous* body fluid. This experiment was repeated eight times with the same results. In the control, samples of coelomic fluid were treated with sea water equal in volume to the sample of anticoagulant and upon standing, both phases were seen. Whether such transformation occurs in all echinoderm coelomic fluids in which such cells are found remains to be seen.

Some problems are presented by the present study of echinoderm coelomic fluid which may be of special interest to comparative and cellular physiologists. The function of the echinochrome-containing eleocytes and the various types of amebocytes still remains a challenge. The function of the vibratile corpuscles of the sea urchins, with the possibility that they represent parasites, is another example of an intriguing problem. The bladder amebocytes and the explosive amebocytes should serve as interesting material for a further study of ameboid movement. The mechanism of the transformation of bladder amebocytes to filiform amebocytes offers still another perplexing problem.

The data so far gathered do not permit evolutionary speculations concerning the origin and diversification of the different types of coelomocytes. However, it cannot escape mention that a greater diversity of cell types appears in the body fluid of the more highly specialized forms, such as the echinoids, than in the

asteroids. A more complete survey of the coelomic corpuscles of other species of each class, especially of the classes studied sparingly at present, may yield information making possible more generalizations than can be made now.

SUMMARY

1. The cellular elements from the body fluid of 15 different species of echinoderms were studied by phase contrast microscopy. Thirteen types of corpuscular elements were identified and the distribution, properties, characteristics and, where possible, functions, were determined.

2. Some types of coelomocytes were observed in the species examined here which had not been previously described, *e.g.* the red corpuscles of the sand dollar and crinoid, and the lobular corpuscles of the crinoid. Some of the coelomocytes formerly described were also found in the species described. Among these are the controversial bladder amebocytes in which the presence of bladder has been questioned. Present studies verify the bladders as real structures easily seen in three dimensions. The bladder amebocyte undergoes a transformation into the filiform amebocyte which represents a pre-coagulation change.

3. A greater diversity of cell types was observed in the body fluid of the more highly specialized forms such as the echinoids than in the less specialized asteroids.

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THE ROLE OF THE BLOOD IN THE TRANSPORTATION OF STRONTIUM^{90}–YTTRIUM^{90} IN TELEOST FISH [1, 2]

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As the result of global fallout and the introduction of radioactive wastes from nuclear reactor plants into the oceans, marine organisms are being subjected to an environment which is potentially hazardous to themselves and to other members of the ecosystems involved. During the last few years, a study has been made in this laboratory of various aspects of the metabolism of radiostrontium by marine fish. These fish may pick up strontium directly from sea water, by way of the skin, gills, or by swallowing the water (Boroughs, Townsley and Hiatt, 1956). They may also take up this element from their food. In any event, the transportation of strontium within the fish, including its excretion, depends upon its transportation by the blood, except for the strontium which is unabsorbed from the digestive tract.

It is the purpose of this paper to report on certain aspects of the transportation of strontium^{90}-yttrium^{90} in teleost blood.

Materials and Methods

The species used in this experiment was *Tilapia mossambica*, a teleost fish. Individuals weighed between 50 and 110 grams each. They were kept in tanks supplied with running sea water.

Two concentrations of Oak Ridge Sr^{90}-Y^{90} were prepared by dilution with saline solution approximately isotonic with *Tilapia* blood. Those fish which were to be bled a day or more after injection were given 100μc of Sr^{90}, while the fish killed at shorter time intervals were given only 10 μc. In both instances the dose injected was 0.2 ml.

The injections were made, and blood was withdrawn with the fishes' opercula in water. Separate fish were used for each time interval studied instead of using a single fish for repetitive bleedings. All the fish were handled as gently and uniformly as possible, and their eyes were covered with the hand. We believe this procedure results in a minimum of trauma.

The Sr^{90}-Y^{90} dose was injected directly into the ventricle of the heart. At predetermined time intervals of 5, 15, 30, and 45 minutes and 1, 4, and 8 days, as much as possible of each fish's blood was withdrawn through the kidney sinus. A red blood cell count was made each time a fish was injected and again when blood was removed.

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[2] This work was supported in part by contract No. AT(04-3)-56 between the U. S. Atomic Energy Commission and the University of Hawaii.

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Immediately after removing the blood from the fish, triplicate 0.1-ml. samples were pipetted onto circles of one thickness of absorbent tissue on aluminum planchettes. Three-tenths-ml. aliquots of the remaining blood were centrifuged for 10 minutes at 2100 rpm in calibrated small bore hematocrit tubes in an International clinical centrifuge. The separated blood in one tube was used for measuring the radioactivity in the plasma and also that associated with the cells. From a second tube the plasma was removed without disturbing the packed cells. Five-hundredths ml. of these cells were washed by re-suspending them twice in fresh saline solutions. All the saline washings were pooled. In a third tube, the same volume of saline-washed cells was lysed with distilled water. The ghosts were washed with distilled water until no further radioactivity could be removed from them. The lysing solution containing the cell contents was added to the distilled water wash for measurement of the radioactivity of the cells exclusive of that bound to the stroma.

Separated organs and tissues were ashed and prepared for counting as previously described (Boroughs, Townsley and Hiatt, 1956). Radioactivity was measured with a thin window G-M tube using a commercial scaler. Counts were corrected for coincidence whenever necessary.

In order to get an approximation of mixing time, Sr^{85} was injected in the heart. Ten, 20, and 30 minutes later, blood was removed from the ventral aorta and from the kidney sinus, and 0.1-ml. samples were counted in a well scintillation counter with the aid of a single channel pulse height analyzer.

Results and Discussion

Preliminary experiments

Since very little is known about fish blood, we were at the outset faced with problems which were not pertinent to the main idea of this research. The first problem to be overcome was the bleeding, because apparently very few biologists have successfully removed blood directly from teleost fish (Prosser, personal communication). In general, fish have been bled by cutting the tail and allowing the blood to drip. Even more refined methods have involved the use of heparin, citrate, or other anticoagulants. We have found it difficult to withdraw unclotted blood from *Tilapia* if the fish had been kept out of water for even a short time. There is probably a dehydration of the blood in some species of fish as a result of asphyxiation (Hall, Gray and Lepkovsky, 1926). If *Tilapia* were stressed by prolonged chasing with a net, by rough handling or by repeated bleeding, removal of blood was difficult even though they were not taken from the water. The cell/plasma ratio increased as it did with asphyxiation.

We had previously observed red blood cell counts which varied between 1 and $4 \times 10^6/mm^3$ in this species of fish, and other workers (Young, 1949) have observed similar large variations with other teleost fishes. Table I is a summary of the rbc counts of the fish used in this experiment and shows that these variations are not intrinsic and that it is possible to remove fish blood that has a reasonably small fluctuation in the rbc count. This blood does not clot even on prolonged standing at room temperature.

The tremendous shift in the number of red blood cells observed in fish blood

TABLE I

Red blood cell count in Tilapia mossambica

Time interval between injection and killing	RBC/mm.³ of blood	
	Counted before dose injected	Counted before blood withdrawn
5 min.	1.444×10^6	1.627×10^6
5 min.	1.150×10^6	1.423×10^6
5 min.	1.375×10^6	1.400×10^6
15 min.	1.350×10^6	2.050×10^6
15 min.	1.209×10^6	1.374×10^6
30 min.	1.548×10^6	1.525×10^6
30 min.	1.175×10^6	1.400×10^6
45 min.	1.125×10^6	1.460×10^6
1 hr.	1.200×10^6	1.600×10^6
1 hr.	1.162×10^6	1.384×10^6
1 hr.	1.050×10^6	1.025×10^6
2 hr.	1.223×10^6	1.347×10^6
2 hr.	1.148×10^6	1.326×10^6
2 hr.	1.150×10^6	1.220×10^6
4 hr.	1.151×10^6	1.169×10^6
4 hr.	1.209×10^6	1.137×10^6
8 hr.	1.199×10^6	—
8 hr.	1.011×10^6	1.102×10^6
1 day	1.312×10^6	1.396×10^6
1 day	1.649×10^6	1.598×10^6
2 days	1.100×10^6	1.199×10^6
2 days	1.298×10^6	1.298×10^6
4 days	1.103×10^6	—
4 days	1.271×10^6	1.362×10^6
8 days	1.150×10^6	1.273×10^6
8 days	1.018×10^6	1.175×10^6

could mean that the plasma, or some portion of it, either leaves the circulatory system or is in effect removed by some pocketing device. The increase in red blood cells may also result from the introduction into the blood stream of cells previously sequestered in an organ or tissue. Studies on fish blood volume and mixing time using either classical techniques or radioisotopes would be of little value if the fish were stressed.

The circulation of fish blood is distinguished from that of higher animals in that oxygenated blood does not necessarily return to the heart. All the blood from the heart goes to the gills, but from the gills the blood may go to the head,

TABLE II

The mixing time of Tilapia blood

Blood source	Minutes elapsed	Counts/min.
Ventral aorta	10	249
Ventral aorta	20	79
Ventral aorta	30	51
Kidney	10	40
Kidney	20	45
Kidney	30	50

Dose: 8477 cpm in 0.2 ml. injected into ventricle of heart.

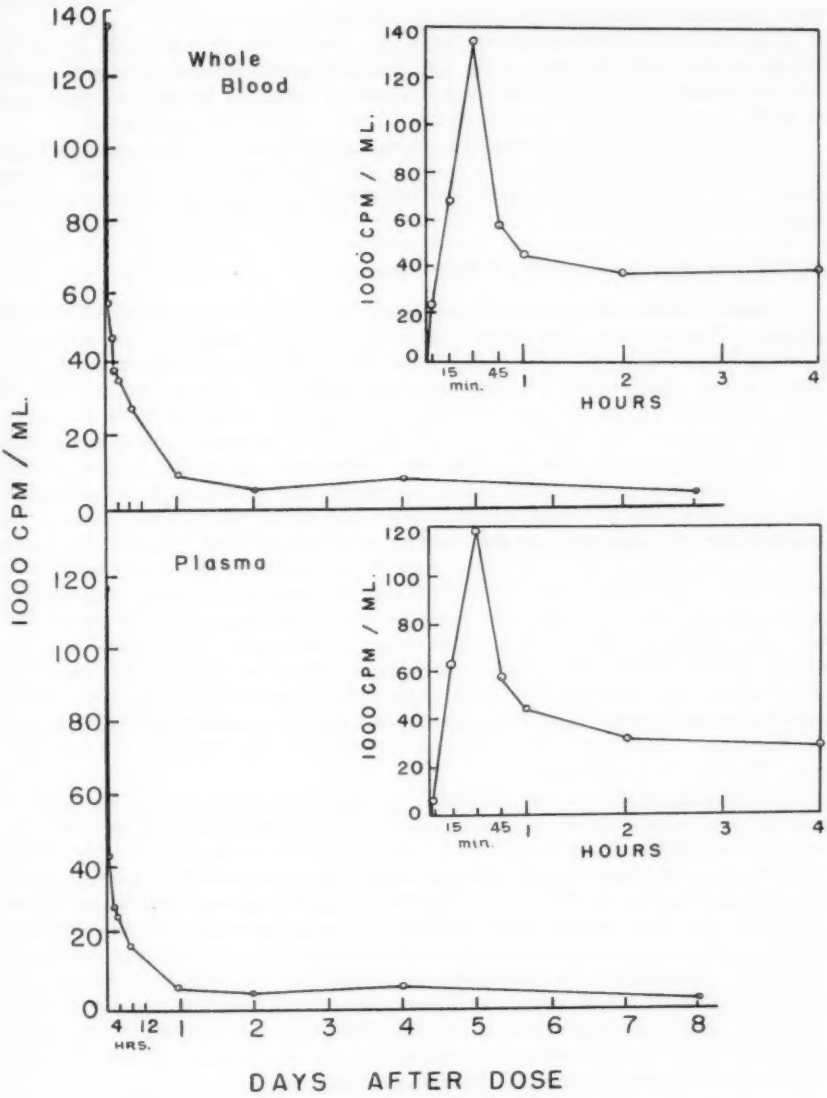

FIGURE 1. The disappearance of Sr^{90}-Y^{90} from the whole blood and plasma of *Tilapia mossambica*.

back to the heart, or to the remainder of the body. This means that mixing is a more complicated process in fish than it is in the higher animals.

The results of studying mixing time in a single fish are shown in Table II. It can be seen that the bulk of the Sr^{85} injected into the heart remained in the anterior portion of the fish, and that it required about 30 minutes for the blood from the ventral aorta and that from the kidney to reach the same level.

Since we lack precise information about blood volume, we have assumed that it is roughly proportional to body weight. We have done this not only on the basis of our own work, but because Martin (1950) has suggested a similar relationship for other teleost fishes.

Rate of disappearance of Sr^{90}-Y^{90} from the blood

Figure 1 shows the rate of disappearance of Sr^{90}-Y^{90} from whole blood and plasma. The numbers have been corrected for body weight. The activity is given in counts/min./ml. whole blood and cpm in the plasma present in 1 ml. of whole blood. Each point on the curve represents the average activity from at least two fish. It can be seen that practically all the radioactivity in the whole blood is carried in the plasma, and that the formed elements can be responsible for only a very small amount. The two curves are practically superimposable. The small inserts on this graph show the appearance of radioactivity during the first few hours, and the larger graph extends the curves to 8 days. Since all the radioactivity was injected into the heart at zero time, at first glance it may seem odd that the amount of radioactivity recoverable from the blood *increases* up to 30 minutes. However, Table II indicates that this apparent increase is a reflection of the mixing time. At least two processes are occurring during this time which make it extremely difficult to find out exactly how much radioactivity is in the blood system. First, the isotopes are being excreted as soon as they appear in the blood, at first principally by way of the gills. Second, radioactivity is rapidly accreted by the various organs and tissues, and thus the concentration is decreasing continuously. We would like to emphasize that it is the resultant of these processes that is being measured.

The radioactivity was very rapidly lost from the blood during the next 30 minutes, and after 24 hours, only between 0.8 and 1.6 per cent of the injected dose remained in the blood, assuming a blood volume of 2–4 per cent of the body weight. The shape of the curves shows that more than one rate process is involved in the disappearance of the radioactivity from the blood. It must be emphasized at this point that the above samples were counted at least three weeks after the fish was killed, so that we were observing the radioactivity in an equilibrium mixture of Sr^{90}-Y^{90}. Strontium^{90} has a half-life of about 28 years and a maximum beta energy of 0.61 Mev. It decays to form radioactive Y^{90} which has a half-life of 2.54 days and a maximum beta energy of 2.18 Mev. Secular equilibrium exists when the Y^{90} decays as fast as it is formed, and the radioactivity of such a mixture is the sum of the radioactivity of the separate isotopes.

In an equilibrium mixture, therefore, no decay of radioactivity would be observable during this experiment unless the two isotopes were separated by either biological or physico-chemical processes. Such a fractionation can be detected by following the counting rate of a sample daily. No changes in this rate will be

observed if no fractionation has occurred. If the rate increases, Y^{90} has been removed and is building up to its equilibrium value at which point it will level off. If the rate decreases, the bulk of the radioactivity must be due to the Y^{90} which is decaying, and the counts will decrease until a level is reached which is a function of the amount of Sr^{90} present.

The role of the blood fractions in the transport of Sr^{90}-Y^{90}

The increase in the counts/minute of the whole blood and plasma in Figure 2 is due to the build up of Y^{90}. There are two simple explanations for the loss of yttrium from the blood. One is that the yttrium was lost prior to its appearance in the blood initially, that is, adsorbed to the glassware used in making the dilutions

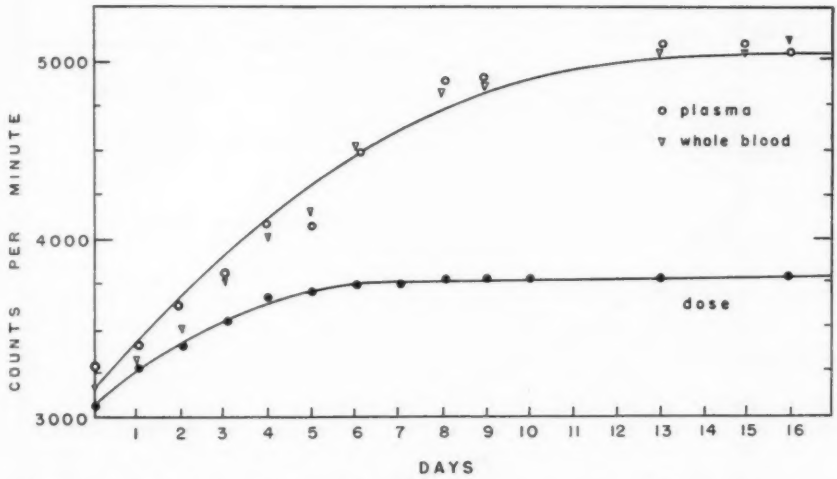

FIGURE 2. The increase with time of radioactivity in samples of whole blood, plasma, and the dose, indicating the build-up of Y^{90}.

and injections. The second explanation is that the yttrium was lost to various organs and tissues through which the blood passed. These explanations are not mutually exclusive and we believe that both processes occur.

In Figure 3, the curve labelled "dose" was obtained by counting planchettes prepared from the Sr^{90}-Y^{90} present in the syringe used for injections. It can be seen that over a period of time, the cpm increased, indicating that some Y^{90} was lost from the equilibrium mixture. This Y^{90} was lost to the glassware. The curve for whole blood and plasma, however, increased to a much higher value, indicating that additional Y^{90} had been removed after the dose was injected.

Figure 3 shows the rate of radioactive decay of the washed and unwashed cells, the saline washings, the washed ghosts, and the distilled water washings which include the cell contents. The decay of the unwashed cells suggests that both Sr^{90} and Y^{90} were associated with the cells. The decay of the washed cells, saline

wash, and ghosts, however, suggests that the Sr^{90} is readily removable either from or through the cell wall. The activity remaining in the washed cells and ghosts indicates it to be Y^{90}, because the decay rates are very similar to the rate for pure Y^{90}. All these conclusions are in harmony with the findings of Thomas *et al.* (Thomas, Litovitz, Rubin and Geschickter, 1950), who showed that radiocalcium, metabolically similar to strontium, was carried in the plasma of rabbit blood.

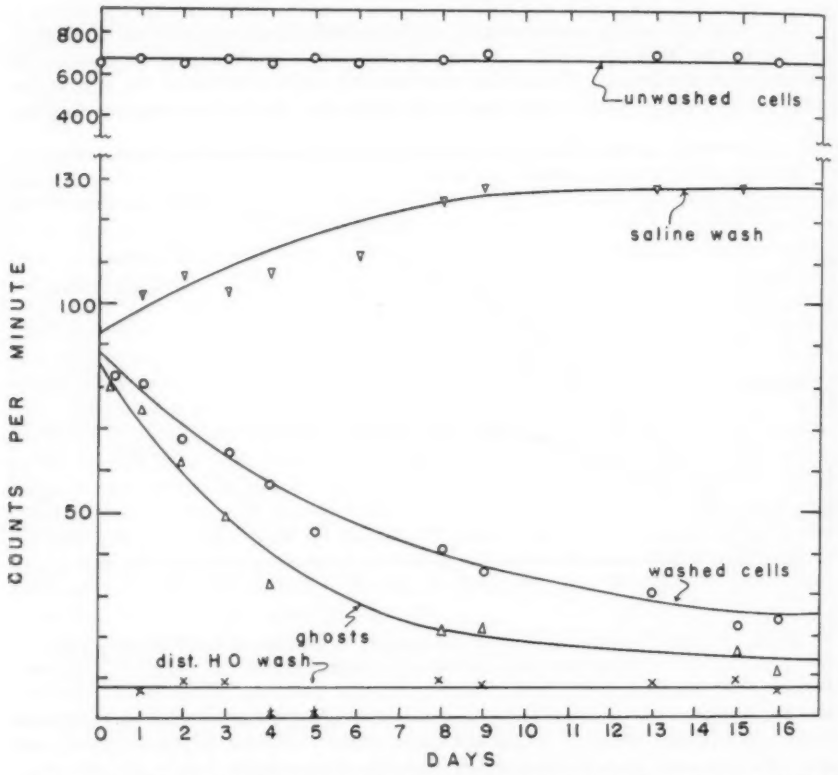

FIGURE 3. The radioactive decay of washed and unwashed cells, the saline and distilled water wash, and the cell ghosts. The decay of washed cells and ghosts indicates that they pick up Y^{90} rather than Sr^{90}.

Retention and distribution of Sr^{90}-Y^{90}

Figure 4 shows the retention by the fish of the injected Sr^{90}-Y^{90} as a function of time. The upper curve represents the entire fish, and the other curves represent, respectively, the bone, integument, gills, muscle, and visceral organs. Each point is the average of at least two fish, and the samples were counted at secular equilibrium. These results may be compared with those obtained previously by

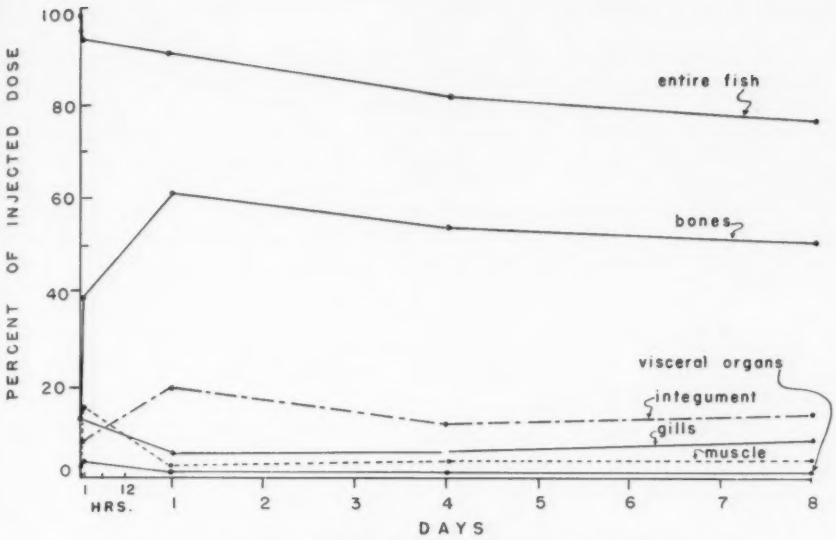

FIGURE 4. The internal distribution of Sr^{90}–Y^{90} injected in the heart of *Tilapia mossambica.*

Boroughs *et al.* on the retention of Sr^{89} by *Tilapia* following ingestion or intramuscular injection. In all instances, the rank order of the percentage of dose retained is the same as that in Figure 4, indicating that once the strontium is absorbed by the blood system, its internal distribution is the same.

Table III emphasizes the rapidity with which the radioactivity appears in the various tissues, including the bones. A small amount of vascular tissue and blood is dissected with the bones, but this additional radioactivity is obviously a very small percentage of the total in the bones. This appearance and retention in the bones cannot be due to accretion by growth, but must represent simply an exchange reaction.

TABLE III

Retention and internal distribution of Sr^{90}–Y^{90} after intracardial injection

Time interval	% injected dose remaining (samples at secular equilibrium)					
	Total of fish*	Bones	Integument	Gills	Muscle	Visceral organs
5 min.	98.2	13.2	13.4	13.4	3.1	3.4
15 min.	95.9					
30 min.	93.2	39.3	8.2	17.3	11.9	4.0
1 day	92.1	61.7	19.2	4.9	4.5	1.4
4 days	81.5	53.7	17.1	4.7	4.2	1.4
8 days	76.6	50.2	16.2	7.4	3.3	1.4

* Including blood.

Biological fractionation of Sr^{90}-Y^{90}

Three fish were injected with Sr^{90}-Y^{90} and killed five minutes, 30 minutes, and one day later. Since the amount of separation of the two isotopes by the glassware was unknown, it is not possible to draw a curve showing the rate of decay of the radioactivity in the various organs that would be a true measure of the decay due to the fractionation by the organs themselves. The planchettes were counted one day after the fish were killed, and this value was taken as a base line. They were then counted until secular equilibrium had been re-established. Table IV shows the percentage increase or decrease in radioactivity in the various organs with respect to the radioactivity present at one day.

TABLE IV

Fractionation of intracardially injected Sr^{90}-Y^{90} by organs and tissues of Tilapia mossambica

Organ or tissue sample	% Decrease of activity 1 day to secular equilibrium	Organ or tissue sample	% Increase of activity 1 day to secular equilibrium
Liver	53.1	Gills	73.8
Gall bladder	42.5	Stomach	22.3
Heart	38.6	Brain	18.3
Kidney	18.8	Muscle	16.3
Spleen	10.6	Intestine	10.4
Gonads	8.5	Eyes	6.9
		Urinary Bladder	5.6
Urine	28.5	Skin	5.0
Blood clots	26.8		
Scales	14.1		
Fat	11.4	Feces	2.1

It can be seen that the first two columns represent the organs which concentrated Y^{90} more than they did Sr^{90}, while the last two columns represent organs that favored the Sr. In general, the more vascular organs and tissues preferred yttrium.

SUMMARY

1. Blood can be easily removed without clotting from the heart or kidney sinus of fishes if the fish are handled gently and their opercula are kept immersed.

2. Blood so removed has a uniform number of red blood cells/mm^3.

3. The mixing time of Sr^{90}-Y^{90} injected in the ventricle of *Tilapia mossambica*, a teleost fish, is approximately 30 minutes.

4. Sr^{90}-Y^{90} rapidly disappears from the blood. At 24 hours, only between 0.8 and 1.6 per cent of the injected dose remains in the blood.

5. The disappearance of radioactivity from the blood depends on more than a single process.

6. Almost all of the Sr^{90} in whole blood is carried by the plasma.

7. Very little Sr^{90} is found either in the cells or on the cell walls.

8. $Yttrium^{90}$, on the other hand, is present in the stroma.

9. The pattern of internal distribution of intravascularly injected Sr^{90}-Y^{90} is the same as that which was found for either intramuscular or oral administration in the same species.

10. Vascularized tissues have a greater avidity for Y^{90} than they have for Sr^{90}.

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DISPERSAL OF THE GELATINOUS COAT MATERIAL OF MELLITA QUINQUIESPERFORATA EGGS BY HOMOLOGOUS SPERM AND SPERM EXTRACTS[1]

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Live sperm or sperm extracts of a number of animal species have been found to possess the property of solubilizing or dispersing the secondary and tertiary envelopes normally surrounding the unfertilized eggs of these species, thus facilitating the approach of the sperm to the egg surface. Groups in which this phenomenon has been demonstrated include amphibians (Hibbard, 1928; Wintrebert, 1929 and 1933), mammals (see reviews by Duran-Reynals, 1942; Meyer, 1947; Meyer and Rapport, 1952), gastropods (Tyler, 1939 and 1948; von Medem, 1942), and bivalves (Berg, 1949). In addition, a number of workers have described the solubilization of the gelatinous coat material (fertilizin) of echinoid eggs by live sperm or sperm extracts. Hartmann *et al.* (1940) extracted the residue of methanol-precipitated seminal fluid of *Arbacia pustulosa* with sea water and reported that the resulting solution was capable of dispersing the gelatinous coat material of unfertilized Arbacia eggs. This extract was also capable of neutralizing the sperm agglutinating property of Arbacia fertilizin, and thus possessed antifertilizin activity. Monroy and Ruffo (1947) described an acid extract of sea urchin sperm which was reported as acting to dissolve the fertilizin of unfertilized eggs. Others have described a decreased viscosity of fertilizin solutions in the presence of live sperm or sperm extracts (Lundblad and Monroy, 1950; Vasseur, 1951; Monroy and Tosi, 1952; Monroy *et al.*, 1954). It has been emphasized (Tyler and O'Melveny, 1941; Krauss, 1950; Monroy and Tosi, 1952; Monroy *et al.*, 1954) that apparent dispersal of the gelatinous coat of unfertilized eggs by sperm or sperm extracts, as well as the decrease in viscosity observed when live sperm or extracts are added to fertilizin solutions, can be accounted for by precipitation of fertilizin by antifertilizin present in the extracts or on the surface of the live sperm. Therefore, any investigation of supposed lytic or dispersing agents from sperm must include experiments which demonstrate that the activity of the agent is separable from the activity of antifertilizin. Ishida (1954) has presented evidence that a fertilizin-dissolving factor is released at fertilization from the sperm of *Hemicentrotus pulcherrimus*. Treatment of the sperm with fertilizin, which rendered the sperm non-fertilizing, did not prevent the solution of the fertilizin coat of the eggs by these sperm. This latter observation tends to eliminate antifertilizin as the agent responsible for removing the fertilizin from the eggs. However, though the sperm concentration employed in the experiments was not stated, sperm carbon dioxide might have been responsible for the solubilizing action

[1] This investigation was supported in part by a research grant (RG 4659) from the National Institutes of Health of the Public Health Service.

of the sperm. Heated (100° C. for one minute) sperm failed to cause dispersal of the fertilizin coat. This failure of heated sperm to cause dispersal of the gelatinous coat has been ascribed, by Ishida, to the denaturation of a dispersing agent on the sperm. Alternatively, the denaturation of respiratory enzymes, resulting in loss of motility and decreased carbon dioxide production, could account for the failure of heated sperm to solubilize the fertilizin of the eggs.

The present report bears evidence that a factor, which is distinct from antifertilizin and which is capable of causing the dispersal of the gelatinous coat of unfertilized eggs, is present in sperm extracts and on the surface of live sperm and of the sand dollar, *Mellita quinquiesperforata*.

Materials and Methods

Animals were collected by dredging on the shallow banks surrounding the University of Florida Marine Laboratory at Sea Horse Key. The animals were transported to Gainesville and kept in the laboratory at 12° C. in sea water supplied with a continuous flow of washed, compressed air. Under these conditions the sand dollars remained alive for approximately two weeks. Eggs and sperm were obtained by injecting the animals with an isotonic KCl solution (Tyler, 1949).

For use in experiments in which live sperm were employed, the "dry" sperm were diluted to a concentration of 5% with filtered sea water. Where separation of sperm from the surrounding fluid was desired, the suspensions were centrifuged at 2900 × gravity in a Servall SS-1 centrifuge for 10 minutes. The supernatant fluids were collected and tested for dispersing activity on the gelatinous coat of fresh unfertilized eggs. The sedimented sperm were suspended in the original volume of fresh sea water and tested for their ability to disperse the gelatinous coat of the eggs.

Sperm extracts were prepared from washed sperm in the following manner. Two volumes of sea water were added to the sperm following centrifugation and removal of seminal fluid, and the resulting 30% suspension was frozen at − 20° C. for 2 to 12 hours. The frozen suspension was then homogenized in an ice bath, using a Potter homogenizer with a motor-driven pestle. After homogenization, the suspension was centrifuged at 11,000 × gravity for 15 minutes in a Servall SS-1 centrifuge. This procedure yielded a gray precipitate, which was discarded, and an opalescent supernatant fluid, which was used as the final sperm extract preparation.

In assaying for the dispersing action of sperm and sperm extracts on the gelatinous coat of the eggs, advantage was taken of the presence of echinochrome granules in the gelatinous coat. As can be seen in Figure 1a, where the outer boundary of the coat has been outlined with antifertilizin, the granules normally have a rather regular position in the gelatinous coat. The locus of this position could be described as a spherical shell lying midway between the outer surface of the gelatinous coat and the surface of the egg. In practice, a small number of freshly shed eggs were transferred with a pipette from the vessel in which they had been allowed to settle, to fresh sea water, and used in the various tests. Dispersal of the egg coat could be followed by noting the length of time required for the echinochrome granules to fall to the bottom of the culture dish, due to the dispersal of the gelatinous matrix in which they were embedded. The time at which the granules were released represented the time at which approximately half the gelatinous coat had been dispersed.

and was taken as the end-point of the reaction. Naturally, every effort was made to insure that the eggs used in the experiments possessed comparable amounts of gelatinous coat material outside the layer of granules. In practice, this was not particularly troublesome since the egg coat of Mellita eggs is quite rigid and not readily soluble in sea water, and since handling of the eggs was restricted to a single transfer to fresh sea water following shedding.

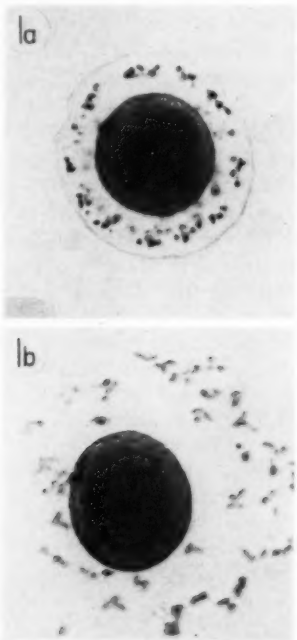

FIGURE 1a. Unfertilized egg of Mellita treated with heated (70° C. for 10 minutes) sperm extract. Final magnification: 175 ×.

FIGURE 1b. Unfertilized egg of Mellita treated with heated sperm extract for 30 minutes, followed by treatment with unheated extract for 15 minutes. Final magnification: 175 ×.

RESULTS

When one drop of 5% sperm suspension was added to one drop of egg suspension (containing about 200 eggs), the gelatinous coat was dispersed in approximately 10 minutes. Elevation of the fertilization membrane did not result in the dispersal of the gelatinous coat of control eggs which were washed and removed to sea water following exposure to the sperm suspension. When the above sperm suspension was centrifuged at 2900 × gravity for 10 minutes, and the supernatant fluid decanted and tested, it was found to be inactive (no dispersal of the gelatinous coat occurred even after 12 hours of exposure to the sperm supernatant). The sedimented sperm, on the other hand, remained able to disperse the gelatinous coat material after being diluted to the original concentration with sea water.

Acidification of a 5% sperm suspension to pH 4, followed by return to pH 8 after 2–4 minutes, with subsequent centrifugation at 2900 × gravity to recover sperm and supernatant fluid, resulted in the loss of the capacity of the sperm to disperse the fertilizin coat. The sperm, in most instances, remained motile following the treatment. The supernatant fluid under these conditions occasionally showed slight antifertilizin activity, as evidenced by the formation of a slight precipitation membrane on the gelatinous coat, but remained inactive with respect to the dispersal of the gelatinous coat. The fertilizing capacity of such acid-treated sperm was reduced, perhaps due to the loss of antifertilizin from the sperm surface (Tyler and O'Melveny, 1941), or to the loss of the ability to disperse the gelatinous coat, or both.

Sperm extracts, prepared as described in the previous section, were also capable of dispersing the gelatinous coat of unfertilized eggs. The final extracts, prepared from frozen-thawed sperm, had a pH varying between 6.9 and 7.1, depending on the particular preparation. Extracts at this pH range were capable of dispersing the gelatinous coat in 30 minutes at 25° C. Control eggs in sea water at pH 6.9 showed no release of echinochrome granules for 6 hours or more. Exposure of active sperm extracts to pH 4 for 3–5 minutes, followed by return to the original pH, inactivated the dispersing factor. The optimum pH for dispersing activity appeared to be 8, since dispersal occurred in approximately 10 minutes at this pH. Alkalinization to pH 9, followed by return to pH 8 after 3–5 minutes, partially inactivated the dispersing factor, dispersal occurring after 30 minutes in these preparations. Heating to 70° C. for two minutes in a water bath completely inactivated the dispersing factor. The heated extracts possessed definite antifertilizin activity (Table I). The heat stability of antifertilizin from sperm of sea urchins (Frank, 1939), and the key-hole limpet (Tyler, 1939) has been previously described. Weak antifertilizin activity was also evident in untreated extracts at pH 8. A faint precipitation membrane appeared on the surface of the fertilizin coat about two

TABLE I

The effect of temperature and pH on dispersing activity of Mellita sperm extract. Activity of extracts assayed at 25° C.

Date of preparation of extract	Exposed to pH	Tested at pH	Dispersal time	Antifertilizin activity
Dec. 13	7	7	30′	±
Dec. 19	7	7	30′	±
Dec. 5	4	8	—	±
Jan. 17	4	8	—	±
Dec. 5	9	8	30′	±
Jan. 3	9	8	30′	±
Jan. 17	8	8	15′	±
Dec. 5	8	8	15′	±
Jan. 3	8	8	10′	±
Dec. 5*	8	8	—	+++
Dec. 19*	8	8	—	+++
Dec. 19*	8	8	—	+++

* Indicates heated extract (70° C. for two minutes).
— Indicates no dispersing activity evident.

minutes after the addition of extract, but disappeared after 5 minutes under the influence of the dispersing factor. This point was further illustrated by experiments with heated extracts possessing stronger antifertilizin activity. The heated extracts (70° C. for 5–10 minutes) formed definite precipitation membranes on the fertilizin coat after two minutes (Fig. 1a). The precipitation membrane so formed did not contract to the egg surface, but remained in the position in which it was originally formed for as long as 24 hours (with no dispersal of the egg coat). Addition of unheated sperm extract at pH 8 caused the disappearance of the precipitation membrane, and, after 10–15 minutes, the dispersal of the gelatinous coat (Fig. 1b).

Experiments involving the addition of extract to eggs up to five minutes prior to the addition of sperm showed that fertilization is not enhanced by this treatment. The fertilization membrane was elevated within three minutes regardless of the presence or absence of the extract. The sperm are apparently supplied with enough dispersing factor to make their way through the gelatinous coat material. Moreover, the gelatinous coat is not dispersed by the dilute suspensions usually employed for insemination. In this respect, the situation parallels that of hyaluronidase of mammalian sperm, in that hyaluronidase added to inseminates does not enhance fertilization (Chang, 1947; Leonard *et al.*, 1947).

The Mellita sperm extracts were not tested on eggs of other species, and the degree of specificity of the dispersing agent is therefore not known at this time. An extract of frozen *Arbacia punctulata* sperm (5% suspension) failed to cause the release of the echinochrome granules of Mellita eggs, but showed strong antifertilizin activity (the precipitation membrane with enclosed granules contracted to the egg surface in 5 minutes). Addition of extract of Mellita sperm, at pH 8.0, caused the gradual disappearance of the precipitation membrane formed by the Arbacia antifertilizin, and release of the echinochrome granules following dispersal of the fertilizin coat. In this connection, it is of interest that a fresh suspension of Mellita sperm was capable of fertilizing eggs treated with Arbacia antifertilizin, in the presence or absence of extract of Mellita sperm, indicating the ability of live sperm to penetrate precipitation membranes.

Further experiments were performed in the hope of discovering the means by which the Mellita sperm extract accomplished the dispersal of the gelatinous coat. A fertilizin solution, prepared by acid (pH 4) treatment of Mellita eggs, with a sperm-agglutination titer of 1 : 1000, was gently shaken with an equal volume of sperm extract at pH 8 for 60 minutes at 25° C. No decrease in titer of the fertilizin solution was evident at the end of the experiment. The sperm extract used in this experiment had been previously shown to disperse the gelatinous coat in 10 minutes, and showed no sperm-agglutinating property. The experiment indicated that no degradation of the fertilizin molecule resulting in loss of agglutinating activity occurred in the presence of the extract. Most probably, the dispersal of the gelatinous coat by sperm extract is accomplished by depolymerization of the gelatinous coat material, and not by splitting of individual fertilizin molecules.

Discussion

Since the dispersing factor is heat-labile under conditions where antifertilizin is stable, it seems reasonable to consider them to be separate substances. This con-

clusion is supported by the failure of strong antifertilizin solutions to cause dispersal of the gelatinous coat, even though a precipitation membrane forms and, in some instances, contracts to the surface. One can distinguish, therefore, between the dispersal of the gelatinous coat and its precipitation. Further, acid treatment of sperm or sperm extracts, a procedure not infrequently used for antifertilizin extraction from whole sperm (Tyler and O'Melveny, 1941), results in the inactivation of the dispersing factor, again indicating that the dispersing factor and antifertilizin are separate substances. Since the respiration of such acid-treated sperm is most probably normal (Tyler and O'Melveny, 1941), carbon dioxide is probably not involved in the dispersal of the gelatinous coat. The observed temperature and pH sensitivity of the dispersing factor suggest that it is protein in nature, possibly an enzyme.

The dispersing factor of Mellita sperm apparently does not act on fertilizin in solution, but only serves to liquify or disperse the gelatinous coat. If the fertilizin, in the gel state, is bound by cross linkages involving the area of the molecule capable of combining with antifertilizin, as Tyler (1948) suggests, the dispersing factor may operate by breaking such cross linkages, thereby releasing fertilizin from the gel. Further, the dissolution of fertilizin-antifertilizin precipitation membranes by extracts containing the dispersing factor may be due to the breaking of linkages at the fertilizin-antifertilizin combining site. Further experiments are necessary before the relationship of the dispersing factor to the fertilizin-antifertilizin reaction can be stated with certainty.

Summary

1. A factor causing the dispersal of the gelatinous coat of Mellita eggs was shown to be present on the surface of Mellita sperm and in frozen-thawed extracts of sperm suspensions.

2. The factor was separable from antifertilizin on the basis of temperature and pH sensitivity.

3. The factor did not degrade fertilizin in solution, but released this substance from the gel surrounding the egg.

4. Active extracts were capable of dissolving fertilizin-antifertilizin precipitation membranes, formed on the surface of the fertilizin coat of unfertilized eggs in the presence of Arbacia or Mellita antifertilizin.

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AN EXOGENOUS REFERENCE-CLOCK FOR PERSISTENT, TEMPERATURE-INDEPENDENT, LABILE, BIOLOGICAL RHYTHMS [1, 2]

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The phenomenon of persistent rhythmicity of one or more of their vital processes is widespread among animals and plants. By persistent rhythmicity is meant that the rhythm still continues when conditions are held constant with respect to all factors generally conceded to influence the organisms.

Reviews of this subject have included those by Bünning (1936, 1956a, 1956b), Jores (1937), Kalmus (1938), Welsh (1938), Park (1940), Kleitman (1949), Calhoun (1944, 1945–46), Korringa (1947), Webb (1950), Caspers (1951), Cloudsley-Thompson (1953), Bruce and Pittendrigh (1957), and Brown (1957d, 1958). The broad distribution of such rhythmicity is suggestive of an hypothesis that all living things have potentially the means of persistent rhythmicity provided it has a period close to that of one of the natural geophysical rhythms. The organismic rhythms usually are essentially temperature-independent in their frequencies, whether the periods are solar-daily, lunar or annual.

Most of the observed rhythms are clearly endogenous, and are labilely adaptable in form and phase relationships to the needs of the organism. Much has been learned, particularly in recent years, as to the properties, including modifiability, of this endogenous rhythmicity. The fundamental problem, however, that of the timing mechanism of the rhythmic periods, has largely eluded any eminently reasonable hypotheses in terms of cell physiology or biochemistry. The problem was already a difficult one when only solar-daily cyclicity was under consideration, but especially in recent years it has been found that one and the same organism may simultaneously possess overt daily and lunar tidal cycles of two bodily processes. Further, the possession of persistent lunar monthly (Brown, Bennett and Webb, 1958) and even annual cycles (Bünning and Müssle, 1951; Bünning and Bauer, 1952; Brown, 1957c) in constant conditions has emphasized the magnitude and complexity of this basic problem.

Added to the property, temperature-independence, in indicating the unconventional character of the rhythm-timing mechanisms, are the repeated demonstrations of the immunity of the frequency-determining mechanism to most metabolic poisons.

Recently, evidence has been rapidly accumulating pointing to the possession by living organisms of basic metabolic cycles of the natural geophysical frequencies,

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produced in the organism by an external cyclic stimulus still operative in so-called laboratory constant conditions (Brown, 1957; Brown, Shriner and Webb, 1957; and Brown, Webb and Macey, 1957). These cycles are not phase- or form-labile. The problem of a common explanation for persistent rhythmicities of all the well-known natural frequencies including the year becomes at once more susceptible to reasonable working hypotheses as to their mechanism when it is firmly established that protoplasm in "constant conditions" is, fundamentally, exogenously rhythmic.

For the study to be reported here, the potato and carrot were selected as organisms neither of which appears to possess any obscuring, labile, endogenous rhythms. It was considered that such organisms would reveal most readily any extant basic protoplasmic cyclicities and also permit easier analysis of any mechanisms they involved.

On the basis of this hypothesis, of an exogenous reference clock providing the timing of cyclic periods, the often-described endogenous rhythms would be considered a consequence of the evolution by the organism of adaptive labile cyclic changes, utilizing the basic exogenous cycle-timing mechanism. The endogenous mechanisms could be inherited. The only inherited aspect of the exogenous cyclicity would be the fundamental protoplasmic responsive systems which are involved.

Materials and Methods

The potatoes, *Solanum tuberosum*, were of the Idaho variety and were purchased from local grocery stores. The carrots, *Daucus carota*, were similarly purchased from local stores. Using a cork-borer, small cylinders, 2.2 cm. in diameter and about 1.5 cm. tall, were prepared from the potatoes in such a manner that each carried an eye on the center of its upper surface. These were permitted to heal their cut surfaces before being set, in shallow water, in respirometer vessels where the same individual organisms were retained up to three or more months. These always gave rise to sprouts and usually also to a root system, and in some instances even developed new tubers up to a centimeter or more in diameter during their sojourn in the respirometers. For the carrots, short cylindrical sections, about the size of the potato-cylinders, were cut and allowed to heal over before being placed in respirometers.

The respirometers have been described earlier. These were originally designed by Brown (1954) and later modified (Brown, 1957a) to permit maintenance of constant pressure.

Five independent barostat-respirometer ensembles, each with 4 respirometers recording as a unit, were in nearly continuous operation during the period of study, Feb. 1, 1956 through Feb. 28, 1958. The potatoes in the respirometers were in constant illumination (estimated at 0.05 ft. c. at the site of the plants) from incandescent lamps supplied by a voltage-regulated line. The temperature, 20° C., was maintained constant by the respirometers being immersed in a large non-stirred, copper water-bath (the barostat) deeply immersed in an outer, stirred, steel (55-gal. drum) water bath, with the latter cycling with a few-minute period within a $\pm$ 0.05° C. range. The pressure was kept constant, 28.5 in. Hg, through hermetically sealing the respirometer-recorder-containing barostat and then aspirating the system to this level. Oxygen and CO_2 tensions were maintained essentially constant through

use of the principle of continuous O_2-replacement together with CO_2 absorbents, and there were clearly no regularly cyclic fluctuations in these substances. Also, the sealed, water-included systems allowed for no changes in humidity.

With a single exception (12 days) the copper tanks, or barostats, remained sealed for periods ranging from 2 to 8 days, with an average of 4.46 days. At these intervals the organisms were exposed for 15–20 minutes to laboratory conditions which were relatively constant over the year. No work was done within 15 feet of outside windows; the laboratory fluorescent illumination at table top was about 45 ft. c. (The carrot study was carried out wholly in a dark-room without any natural illumination.) The room temperature was relatively constant, about 75° F., except for slightly higher values during the summer months. The barostats were opened at various hours of the day from 8 AM to 10 PM. Excluding those days the respirometers were opened to renew the O_2 and the CO_2 absorbent, a total of 2485 uninterrupted calendar days of data were obtained.

The recording systems of the respirometers possessed two points of slight mechanical frictional resistance, a) a two-point pivotal, spring-scale bearing, and b) the point of contact of the ink-writing pen with the slowly moving paper. These resulted in random, spurious apparent intra-hour fluctuations in rate of O_2-consumption. Since the principle of operation of the recorder was one with which the hourly values of O_2-consumption were obtained by calculating the differences between consecutive hourly markers on a continuing trend-line denoting cumulative O_2-consumption, these spurious fluctuations in apparent rate could, and undoubtedly did, produce larger hour-to-hour differences than bore any significance. Hence, time units of less than three hours (three-hour "moving means") were never used in determining the mean rates centered on any given hour. By this means the random mechanically induced error was reduced to about one-third its single-hour influence. For most of the study reported here, a weighted (1:2:3:3:3:2:1) seven-hour "moving mean" was used. This reduced by essentially 90% the random fluctuations while retaining all the precision of measurement of average, actual, O_2-consumption for this longer interval, as modified by its weighted character. The shorter period, three-hour, means were found necessary, however, to expose the relationship between day-by-day 6 AM deviations in O_2-consumption from daily linear trends and the concurrent day-by-day mean rates of barometric pressure change for the 2–6 AM interval. Although some clearly significant short-period fluctuations were obscured, therefore, by the seven-hour weighted "moving means," these were considered superior to the shorter periods for the accurate description of the general characters of the longer-period, daily and annual cycles to be described herein.

The records for the five completely independent, respirometer-recording systems were first dealt with individually and three-hour and weighted seven-hour "moving means" were prepared month by month for the period of study. From the latter values were calculated the mean daily rates of O_2-consumption and the data were then converted into hourly deviations from the solar daily means. The number of uninterrupted days of data from the 24 months of study ranged from 93 to 129 each month. The hourly deviations for all the respirometers operating were averaged for each calendar day, and these average daily cycles then converted to hourly deviations from a 1 AM to 12 midnight linear trend-line. This will be referred to as the deviations from linear daily trend. From these data the forms of the mean

daily cycles for each month were obtained. The slope of this linear trend-line itself shows apparent monthly and annual periodisms which have been treated elsewhere (Brown, 1957c; Brown, Bennett and Webb, 1958). The trend involved a mean daily increase during the two-year study of 6.7%, and included, as a large

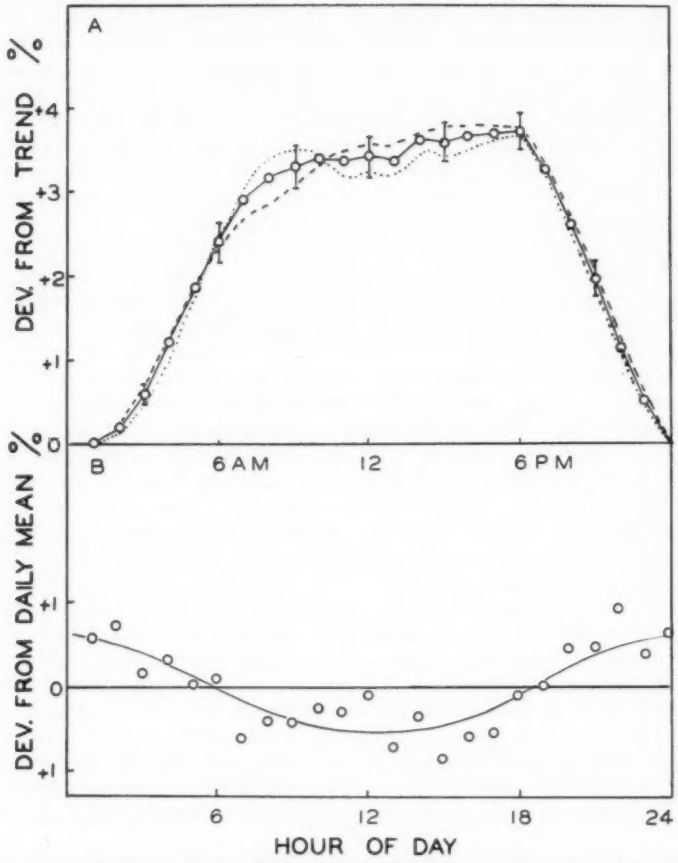

FIGURE 1. A. The mean solar-day cycle of O_2-consumption in the potato (solid line) with standard errors for selected hours. This is expressed as % deviations from linear daily trend. The dashed curve is the cycle for the first year of study, the dotted curve, for the second. B. The mean apparent sidereal-day cycle of the potato for the two-year period of study.

component, the apparent smoothly gradual recovery over a 3- to 5-day period, from the inhibitory influence of the room-illumination intensity. The mechanical recording system, itself, departed from linearity over its total range by 10%, departing in such a direction that there would be expected on this basis an average of about 2% increase per day.

An entirely independent and parallel study was made of O_2-consumption of the sections of the carrots, for the 8-month period Oct. 1, 1956 through May 31, 1957. Two respirometer-barostat ensembles were employed for the first three months, and four for the remaining five months. These were maintained in darkness in a photographic darkroom about 60 feet away from the place of the potato study, but similarly on the ground floor of Cresap Biological Laboratory, a three-story steel and mortar building. The respirometers were maintained and the data processed by a person not involved until the termination of the carrot study in the paralleling and continuing potato study.

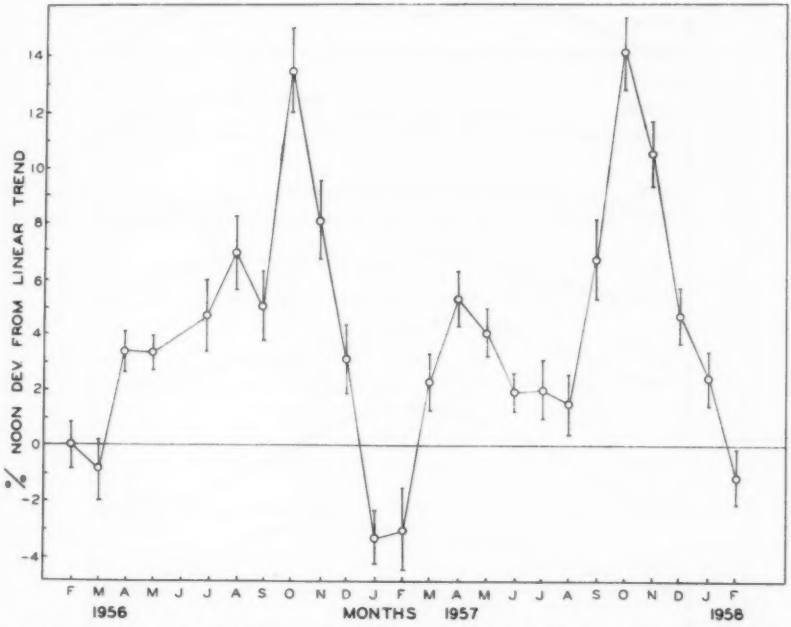

FIGURE 2. The relationship between average % noon deviation in O_2-consumption in the potato from linear daily trend and month of year during the 25-month study. Standard errors of means are shown.

RESULTS

During the period, Feb. 1, 1956 through Feb. 28, 1958, in the study of the potato, the only days omitted were May 25, the month of June and the first three days of July, 1956, and October 4, 1957.

The form of the mean daily deviation from trend, expressed as percentage of the daily mean rate, is shown, with the standard errors of arbitrarily selected mean values, in the solid-line curve of Figure 1, A. The errors of the other values are quite comparable in size. Superimposed on this are the mean cycles for each of the two years separately: Feb. 1, 1956 through Jan. 31, 1957 (the dashed curve) and

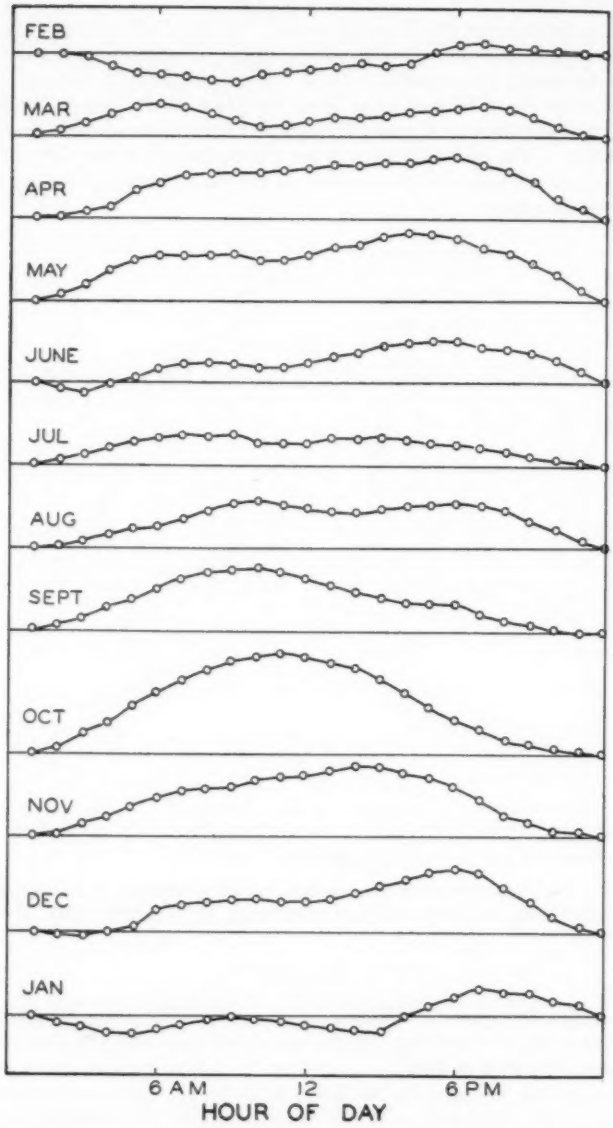

FIGURE 3. The forms of the average daily cycles for each month of the year obtained in the two-year study. The ordinate values are deviations in comparable arbitrary units.

Feb. 1, 1957 through Jan. 31, 1958 (the dotted curve). The average amplitude of the daily cycle was clearly quite reproducible for the two years at about 3.7%. There was also clear suggestion, in the skewed cycle form, of a bimodality with morning and afternoon maxima, a condition more conspicuous for the second than for the first year of study. The mean sidereal-day cycle (23 hours, 56.07 minutes) for the two-year period is shown in Figure 1, B. This was obtained by displacing

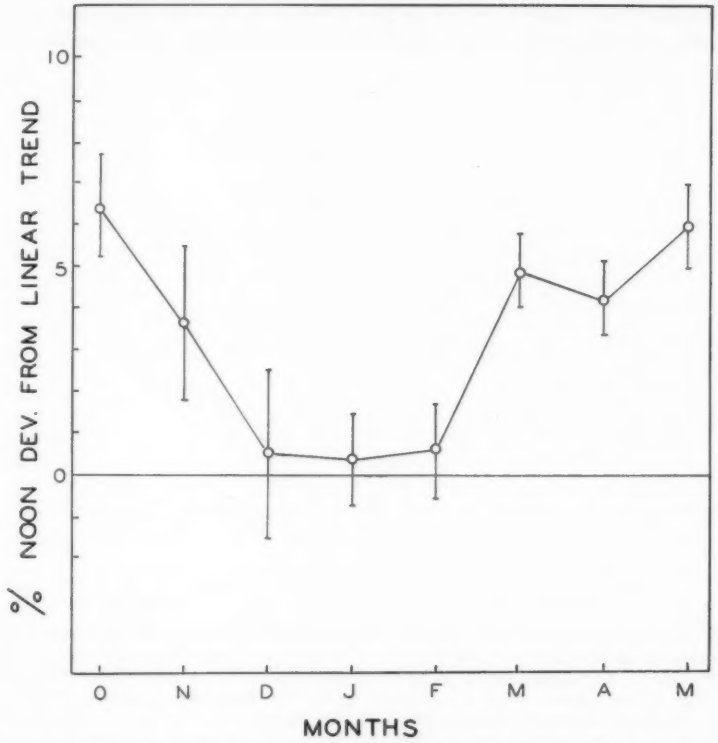

FIGURE 4. The relationship between the noon deviations in O_2-consumption from linear daily trend and calendar month in the *carrot* during an 8-month study. The standard errors are depicted.

the consecutive mean monthly solar-day cycles each by two hours to the right during the two-year period, to bring into reasonably close synchrony ($\pm$ 1 hour) the hours of the sidereal day. The numbered hours are fixed by the solar-day hours of the first month, February, 1956. This process also randomizes daily trend.

The form and amplitude of the solar daily cycle showed differences from month to month which revealed that it was undergoing a modulation of an annual frequency. This was quite evident when one used, for example, the parameter of average monthly noon deviation, in percentage, from 1 AM to midnight daily

linear trend. The deviations, month-by-month, for the period Feb. 1, 1956 through Feb. 28, 1958, together with their standard errors, are depicted in Figure 2. These indicate minimum annual values, involving often even apparent cycle inversion, during the coldest months of the year and a major maximum in the month of October. A lesser, or incipient, maximum occurred in April–May. The maximum range is seen to extend from − 3.4% to + 14.2%.

An annual cycle in over-all form of the mean daily cycles for the months of the year is evident in Figure 3, where twelve average cycles, the means for two years, have been plotted in terms of average deviation in arbitrary units from linear-trend.

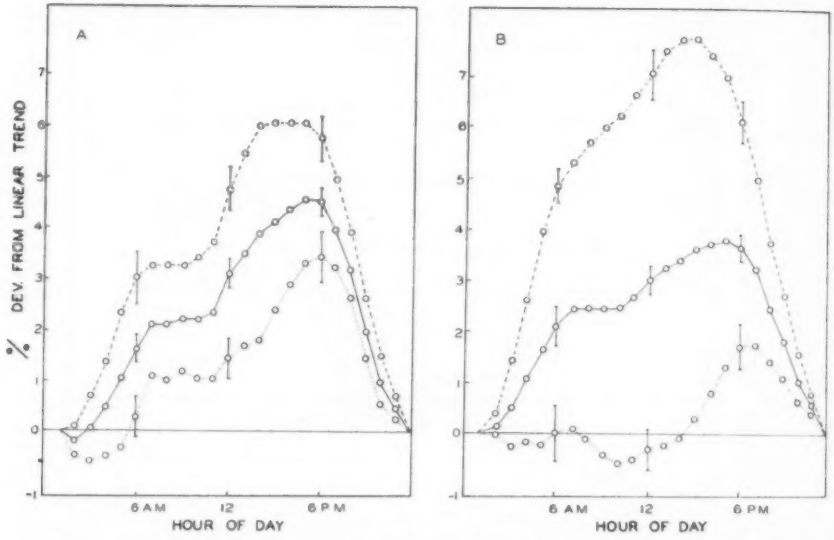

FIGURE 5. A comparison, for the same 8-month period of study, of the forms of the mean daily cycles for carrots (A) and potatoes (B). Solid curves show the mean cycles for the whole 8-month period. The dashed curves show the average cycles for October, November, April, and May. The dotted curves show the average cycles for December, January, February, and March. Standard errors of selected times of day are shown.

Although these data are not expressed as percentage deviations, they do illustrate the gradually-changing form of the cycles from unimodality with essential inversion in February, but with a 7 PM maximum, through a period of bimodality with the two daily maxima gradually converging towards noon to reach unimodality with a maximum at 11 AM in October. Thereafter, bimodality reappears and continues, becoming only feebly evident as an apparent residual in the essentially unimodal inverted cycle of January which like the succeeding month, February, has a 7 PM maximum.

The study of the carrot revealed striking similarity of its major mean cycles with those of the potato. Figure 4 shows the mean % noon deviation from linear trend for each of the eight months. Like the results obtained with the potato for

the same calendar period, this passed from an early-fall higher value, through a winter minimum and back to a higher spring value. Fewer data were available during the first three months, hence the errors were larger. The range was less than for the potatoes. Figure 5, A and B solid curves, compares the mean 8-month daily cycles for the carrot and potato, and the average cycles for the two fall and two spring months (dashed curves) as compared with those for the four intervening colder months (dotted curves), The similarities of these two widely different kinds of plants and plant portions (roots vs. stems) for the same periods, in the %

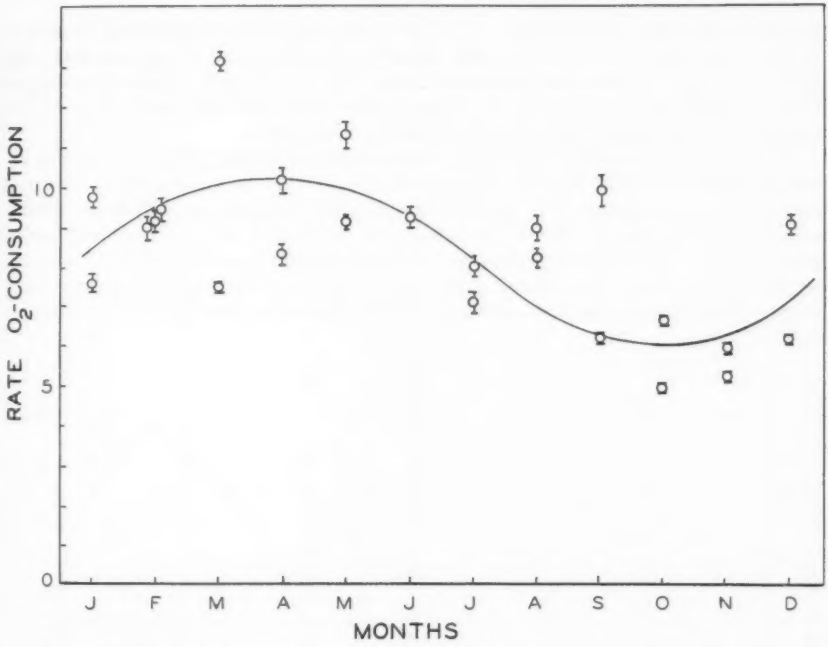

FIGURE 6. The relationship between mean rates of O_2-consumption and each of the 24 months studied during 1956, 1957, and 1958, and time of year. Standard errors of the means are indicated.

amplitudes of the fluctuations, in the times and the changing times with time of year of the primary maxima, and in the times of secondary, or incipient, maxima, are strikingly apparent from the figures.

A second kind of annual cycle appears also present in the data. This is in the mean daily metabolic rates. In Figure 6, are to be found the mean monthly rates of O_2-consumption, in arbitrary units, for each of the 24 months of study, together with their standard errors. Two conclusions are evident from the figure: (1) The maximum rate of O_2-consumption occurs in the April–May period of the year and minimum rate in October–November. The rate for the former period ap-

TABLE I

Signs of the average monthly correlations of the 6 AM deviations from linear trend with the mean 2–6 AM rate of barometric pressure change

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Year 1		−	−	−	+		+	+	+	+	−	−
Year 2	−	−	+	+	+	+	+	+	+	+	−	−
Year 3	−											

proaches twice that of the latter. (2) The mean rates for corresponding months of the two consecutive years may be quite significantly different from one another, suggestive of a specific, *time-environmental* factor involved in an exogenous regulation. There is nothing in these data to suggest other than that the mean form of this annual cycle will ultimately be found essentially sinusoidal.

In view of the correlations highly significantly different from zero earlier reported (Brown, 1957a) to exist between the 5–6–7 AM mean deviations (without sign) in O_2-consumption from the daily means and the mean 2–6 AM rates of barometric pressure change, this relationship was examined for the two-year period involved here. Three-hour values of O_2-consumption centered on 6 AM were recorded as deviations from linear daily trend, and three-day moving means calculated. These were correlated with comparable three-day moving means of the av-

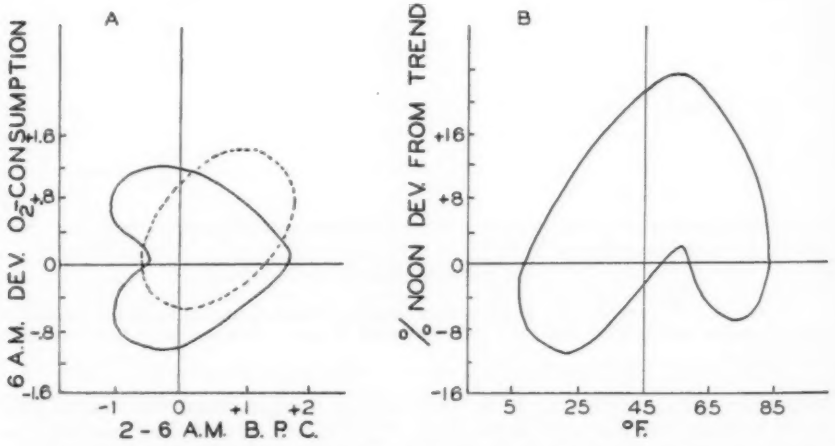

FIGURE 7. A. Solid line: An outline of the general form of the scatterplot between mean 2–6 AM rate of barometric pressure change and the 5–6–7 AM mean deviation of rate O_2-consumption from daily trend for the same day during the "colder" months (see text). Broken line: The same for the "warmer" months. Data for both involve three-day moving means. The two patterns together include 98% of all points. B. An outline of the form of the scatterplot (97% of all points) between % noon deviations from linear trend in potatoes in constant conditions and concurrent outdoor air temperature, taken from data of 149 non-overlapping three-day averages.

erage 2–6 AM rate of barometric pressure change, for the corresponding days. It should be emphasized that only a single value was used for each day for each phenomenon; hence, this did not involve a correlation of parallel daily cyclic trends. A positive coefficient, highly significantly different from zero, was obtained. This correlation, as one would anticipate in view of the essentially aperiodic, large climatic barometric pressure changes, rapidly drops to insignificance as one correlates

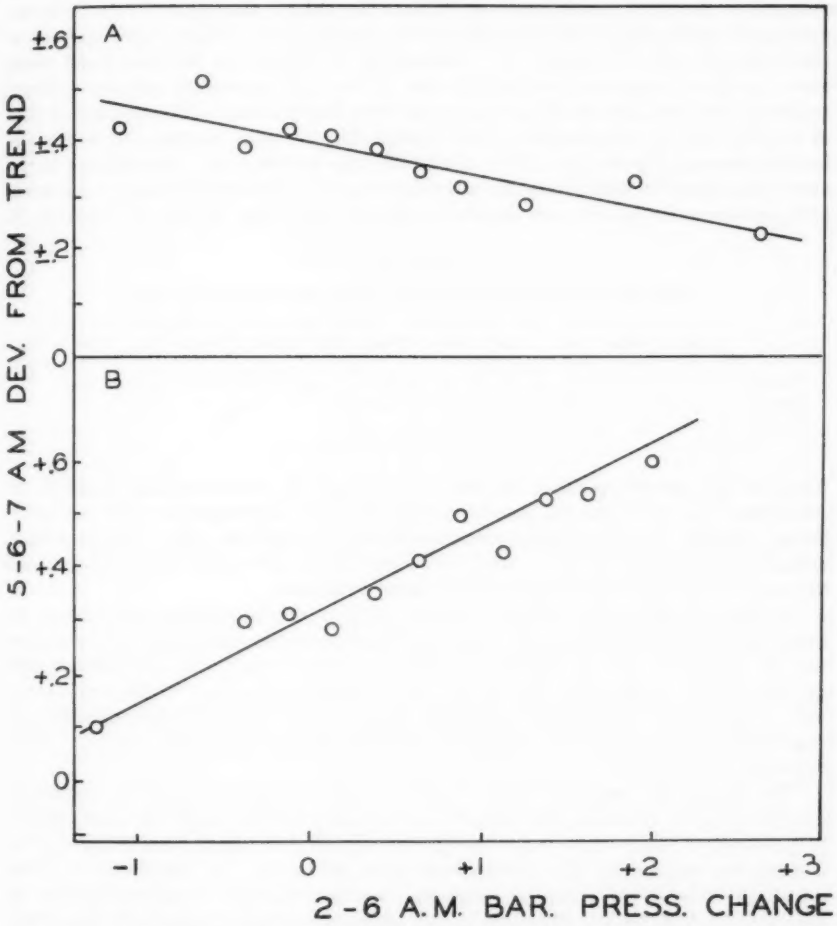

FIGURE 8. The regressional relationship of the average three-hour rate of O_2-consumption of the potato centered on 6 AM, and expressed as deviation from linear daily trend, on the average rate of barometric pressure change during the 2–6 AM interval for the same morning for the colder months of the year (see Table I). $P < 0.005$. B. The relationship comparable to that in A, but for the warmer months of the year (see Table I). $P < 0.001$.

in increasing lag on lead relationships up to two to three days (Brown, 1957a) indicating a direct response of the organisms to some pressure-change-correlated external variable. But this relationship was found to contain a characteristic sign-change twice a year as seen in Table I. In this table, a dash indicates those months in which there was a negative correlation between the rate of the 2–6 AM barometric pressure change and the 6 AM deviation, *without sign*, from linear trend. The form of the scatterplot relationship for the 299 days of this negative period is outlined by the solid curve (encloses 91% of the points) in Figure 7, A. The regressional relationship of the deviation in O_2-consumption, without sign, upon pressure change is seen in Figure 8, A. During the + months, on the other hand, there was a positive correlation between the rate of 2–6 AM barometric pressure change and the 6 AM deviation in O_2-consumption from linear trend. Ninety-one per cent of the 389 days in a scatterplot of the relationship for these months fell within the broken curve of Figure 7, A (98% of all 688 daily points + or − months fell in the areas prescribed by both the solid or broken lines). The regressional relationship of O_2-consumption on pressure for the + months, with sign, is seen in Figure 8, B.

TABLE II

Signs of the average monthly 2–6 PM change in barometric pressure

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Year 1		+	+	+	−	−	−	−	−	−	+	+
Year 2	+	−	+	−	−	−	−	−	−	−	+	+
Year 3	+											

Since in the warmer, positive, months of the year, the overwhelming mass of the deviations was +, it was not possible to find any real difference between the correlations whether the deviations were treated with, or without, sign. In the colder, negative, months, on the other hand, about half of the deviations were negative, and the range of the latter as great as for positive deviations.

In view of the earlier report (Brown, 1957a) of comparable correlations between the 6 PM deviation in O_2-consumption from daily mean values in potatoes, and the afternoon rate of barometric pressure change, and also correlations with the mean daily pressure of the second day thereafter (Brown, Webb and Macey, 1957), the former relationship including signs, it is of interest to compare the annual cycle in the sign of the average 2–6 PM barometric pressure change. These are seen in Table II.

The similarities between Tables I and II suggest that this aspect of organismic annual cyclicity, involving the mean forms of daily cycles, might in some manner be caused by a factor whose daily fluctuation reflects the annual cycle in form of the well-known mean daily tidal atmospheric pressure cycles. In these daily pressure cycles, the time of the morning maximum remains relatively fixed throughout the year at 9–10 AM, but the afternoon, major minimum of the day gradually shifts from about 2 PM in winter to about 7 PM in summer. This last is the basis for the sign changes in Table II. Thus, any pressure-correlated effective external physical factor could provide such an annual cycle in the daily cycles as that described herein.

Another clearly evident correlation is seen in the relationship of the % noon de-

viation from linear trend in the potato to the concurrent outside mean daily air temperatures.[3] A two-year study of the comparison of non-overlapping three-day periods of air-temperature and of noon deviation of the daily cycles in constant conditions yields a scatter plot relationship as illustrated in Figure 7, B. The line includes 97% of the 149 values. The regressional relationship of noon-deviation of the potatoes on temperature (using 5° F. class intervals) is illustrated in Figure 9. The relationship seems adequately described as a linear one, but with a sign change near 57.5° F. Calculation of the coefficient of correlation for noon deviation in

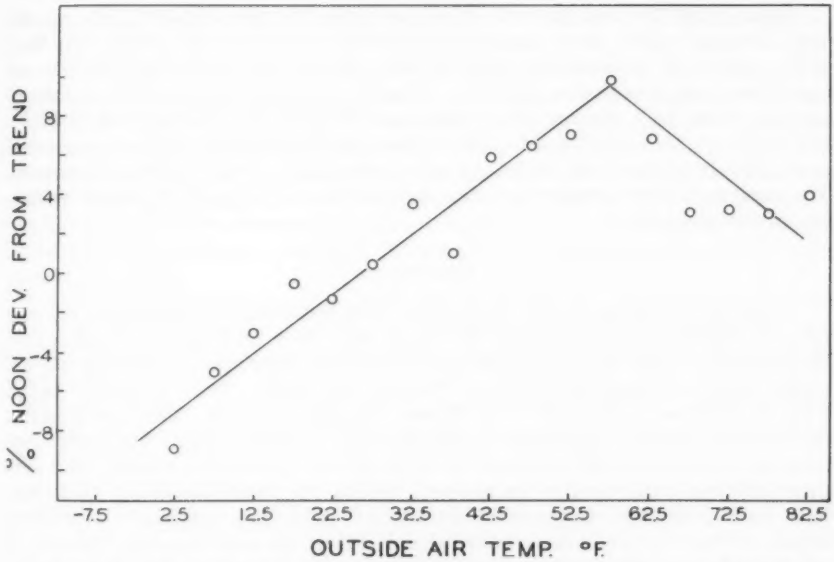

FIGURE 9. The regressional relationship of the noon deviation in O_2-consumption in the potato, expressed as % deviation from daily linear trend on simultaneous outdoor air temperature.

O_2-consumption with the ± deviation in temperature from 57.5° F., yielded a value of -0.51 ± 0.049. This clearly indicates that the external factor responsible for the 24.0-hour cycles of metabolism is correlated in its fluctuations with air temperature, resulting in a condition where a spurious organismic Q_{10} of cycle amplitude of more than 3 could be apparent (*e.g.*, in range 32.5° to 52.5° F.). This provides another piece of information which will probably lead eventually to identification of the still unknown external factor responsible for the organismic basic periodisms.

That the relationship to temperature is rather substantial, is given further support in that the regressional relationship of noon deviation on temperature exhibited a sign change about 57° F. in the first year, 1956–57, just as it did again in the second, 1957–58, despite the fact that in 1956 there was no clear *absolute* summer de-

[3] These data were generously provided to me by the Chicago Office of the U. S. Weather Bureau.

cline in amplitude of the mean monthly cycles (Fig. 2) as was found in 1957. Also, during the winter months the correlations are observed (Fig. 9) to continue in the same linear relationship even at temperatures lower than the lowest mean monthly ones in the two years involved in this correlation (29.4°, 28.7°, 18.8°, 27.3°, and 26.3° F.) which averaged about 26° F. These last facts suggested intra-month significant temperature correlations which were borne out by investigation of the correlations using the data of the five coldest months now expressed as deviations from monthly means. The correlations continued in temperature ranges well exceeding any mean month-to-month difference.

Again, using the data for both of the two years, the transitional months, April, May, October, with a mean temperature of 54.8° F. (47.2, 60.5, 60.8, 49.5, 58.8, 52.0), expressed as deviations from monthly means, the critical temperature for sign change was again quite apparent. Finally, employing the warmest months of the year, June, July, August, and September with a mean temperature of 71.1° F., (72.9, 74.1, 65.0, 71.2, 76.4, 73.4, 64.2), there was a suggestion of the existence of a second sign change with again a positive correlation at the higher temperature. The number of high-temperature days was insufficient, however, to enable resolution of this last point.

Discussion

From the foregoing results it is evident that potatoes, and apparently the carrots too, display a quite reproducible mean solar daily cycle provided adequately long periods of time are used to render random the influences of such modulating longer cycles as a lunar day (Brown, Freeland and Ralph, 1955), a synodic month (Brown, Freeland and Ralph, 1955; Brown, Bennett and Webb, 1958), and, in this report, a low amplitude apparent sidereal day, and an annual cycle. These described mean solar-day cycles are obviously of quite precise 24.0-hour frequency, and adequate evidence is at hand to be assured, beyond all reasonable doubt, that these have their frequency exogenously determined. This last conclusion is assured in part through the well-known knowledge that there are solar-day tidal rhythms of atmospheric pressure, together with the fact that the living organism has access to information of them through its responses to the day-to-day, essentially random, weather-induced, disturbances in their regularity. That the factor influencing the organism is not pressure itself, is evident from the fact that these and other experiments have involved organisms maintained for long periods in constant pressure. The external factor which is involved appears to have its primary action upon the organism at the times correlated with the early-morning rise in barometric pressure and the afternoon fall. These would presumably be the times of most rapid change in physical factors fluctuating with the day-night cycle, and hence be the times of their maximal stimulative effectiveness.

As pointed out earlier in this report, the presence of the well-known annual change in the form of the daily, tidal, barometric pressure cycles, and the described response of the organism in the late afternoon to a pressure-correlated external variable would have led to the prediction of the occurrence of an annual cycle in the form of the daily cycles. Such a prediction has been fulfilled in this study. This adds still further, therefore, to the assurance that the forms of the daily basic metabolic oscillations in living organisms are exogenous.

Since background radiation, too, possesses good mean solar-day cyclicity, and the organism follows the essentially random fluctuations in its cycle amplitudes from day-to-day (Brown, Shriner and Webb, 1957) very safely beyond what would be expected through chance, when and only when contemporary data are correlated, this constitutes a third line of evidence for exogenous origin of mean daily metabolic cycles.

The existence of an annual cycle in the potato in constant illumination, temperature, pressure, etc., was reported earlier for fluctuations in linear daily trend (Brown, 1957c), as were also synodic monthly cycles of this parameter (Brown, Bennett and Webb, 1958). In this paper there is described an apparent annual cycle in basic metabolic rate, a cycle which appears to be of simple sinusoidal character with maximum in April–May and minimum in October–November. This cycle involves an approximate doubling of rate in passing from minimum to maximum values in the annual cycle. Comparable, synodic monthly, cycles in metabolic rate in potatoes (Brown, Bennett and Webb, 1958) involved, as the average during a year of study, about a 15% increase from minimum (new moon) to maximum (third quarter) values. By further comparison, the amplitude of the daily cycles, though undoubtedly artificially depressed through the use of the seven-hour weighted moving means, displayed about a 3.7% increase from midnight minimum to 6 PM maximum values.

The regressional relationship of amplitude of the daily cycles on mean daily temperature for three-day periods (Fig. 9), with its coefficient of determination of about 0.26, and its critical temperature for sign-reversal, together with the earlier barometric-pressure-change reversing correlation, suggests again the exogenous origin of this daily cycle period, and, at least in large measure, also cycle form. This is especially true, since the relationship to temperature seems to persist into the weather-correlated, intra-month, temperature fluctuations.

In examining Figure 9 and noting the relationship of cycle amplitude to temperature, and recalling that the mean daily temperature range is about 16° F., with not very uncommonly single days with ranges up to 25° to 30° F., one is tempted to postulate that the factor that is responsible for transmitting to the organism in "constant conditions" information on outside air-temperature, is, through its temperature-correlated fluctuations alone, contributing importantly to the 24-hour periodic metabolic fluctuations themselves. In support of this hypothesis is the rough similarity in the average forms of the annual fluctuations in the amplitudes of ground-level daily temperature change and metabolic cycles. Both, as average for the two years, showed lowest values in the coldest winter months and highest values in late spring and late summer to early fall, with a summer amplitude reduction. The relationship between these two phenomena is seen in Figure 10. The October peak, so conspicuous for the metabolic cycles, is much less evident for the temperature changes.

For each year this relationship between these two phenomena appeared to trace out general ovoid form. The two-year mean month-by-month relationship is shown by the numerals 1 (January) through 12 (December), and the dotted ovoid curve roughly traces their course. It is interesting to speculate that this difference between the organismic and temperature annual cycles may find its explanation in the changing natural smog content of the atmosphere. The terpenes, volatilized from

plants, polymerized by the ultra violet light from sun, reach a maximum in October (personal communication from Professor F. W. Went). This smog may, through influencing the amount of heat absorption from sunlight, produce in October the highest amplitude daily temperature changes of the year at levels in the atmosphere where temperature changes produce greatest influence upon the factor directly affecting the organism. One process, known to be temperature-dependent, is the rate of spontaneous decay of cosmic-ray-derived mesons. The larger the atmospheric depth involved in the temperature change, in this instance, the larger would be expected the temperature influence.

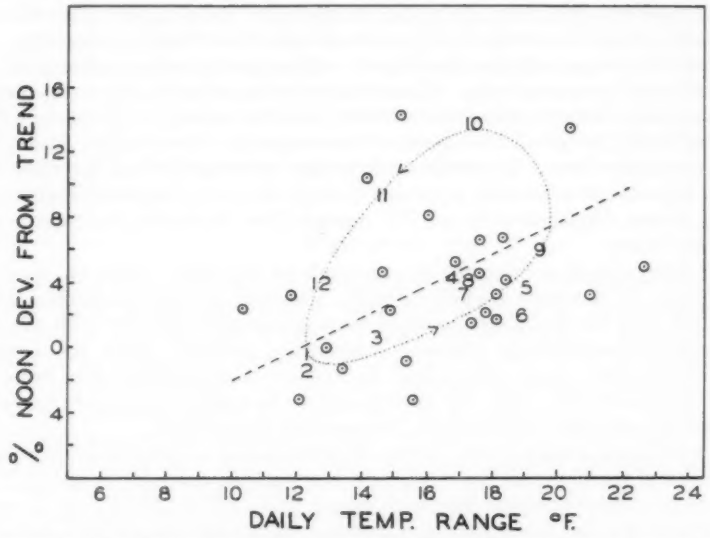

FIGURE 10. The relationship between mean monthly daily temperature ranges and the mean monthly noon deviations in O_2-consumption from daily trend. The Arabic numerals indicate the mean monthly relationships for both of the years, with January as 1, February as 2, March as 3, etc.

Now that it appears true beyond reasonable doubt that the living organism, as part of its cyclic geophysical environment, is exhibiting metabolic cycles with the natural solar day, lunar, and annual periods, the question arises as to what the relationship of these metabolic cycles might be to the well-known endogenous physiological and behavior cycles which have been abundantly described, especially during the past fifty years for species representing the gamut of the animal and plant kingdoms. Those studied experimentally have included especially the sleep-movements of plants, the spontaneous motor activity of numerous animals, physical and chemical adaptation of compound eyes, integumentary color changes, eclosion in flies, learned periodic behavior in insects and lunar-tidal rhythms of color-change and of motor activity. These cycles are clearly endogenous in most instances since the forms and phase relationships of the cycles relative to external physical ones

may be modified by appropriate treatment of the organism and will persist for a few, and often many, cycles. The endogenous cycles also may gradually drift away from their initial phase relationships when placed in constant conditions away from cyclic light and temperature changes. The rate of drift is characteristically a function of the constant illumination, or constant-temperature, level; brighter light behaves usually like higher temperature. But in some other instances the cycles show no measurable drift over long periods, behaving in their precision, therefore, more like the mean metabolic cycles. Also, cycles of quite other periods than the natural daily ones may be directly induced by light, for example, but the organism thereafter placed in constant conditions reverts at once to daily cycles.

The characteristic of essential temperature-independence of the cycle-frequencies, whether one deals with solar-day, lunar, or annual ones, made it rather improbable that the basic frequency-determining mechanism was endogenous. However, one hypothesis that has been rather widely entertained bears the assumption of the possession by the organisms of accurate, fully autonomous, endogenous biological clocks timing all the natural period lengths. Although the majority of investigators have always cautiously dissociated the possible clock mechanism from the endogenous physiological cycles studied and whose frequencies they appeared to regulate, some of the more recent supporters of this hypothesis of an autonomous endogenous clock have uncritically identified the fundamental clock system with each of the various observed endogenous and labile physiological cycles studied. On the basis of this quite unjustified assumption, the postulated clock is then usually considered to have all the properties demonstrable for these clock-regulated, and undoubtedly endogenous, cycles.

None of the several hypotheses suggested for the independent biological clock, however, have satisfactorily resolved the problem of the determination, in an essentially temperature-independent manner, the exact intervals of the turning points in the persisting cycles. So-called endogenous rhythms with single cycles ranging from 24 hours to a year in length cannot readily be conceived in terms of any fully autonomous mechanism based upon the reaction kinetics of any biochemical, or biophysico-chemical, systems with which we are now familiar.

An alternative hypothesis, advanced by Brown (1957b), proposed that the basic mechanism of temperature-independence of the frequency of biological rhythms in "constant conditions" involves the operation of a cyclic exogenous stimulus operating upon a responsive protoplasmic system, and giving rise in all cells to systematic fluctuations containing all the major natural periodisms of the external environment. These could then be readily used by the organism in timing its endogenous cycles. The latter could be considered as bearing any pre-set, fixed lag or lead relationship, or even possess a smoothly and continuously drifting relationship, thus providing endogenous cycles of frequencies differing slightly in lengths from 24 hours. The often-reported small influence of light and temperature level on cycle length in constant conditions can well operate through influencing the coupling mechanism between the exogenous basic clocks and the endogenous organismic rhythms.

We still do not know whether the exogenous daily clocks operate on a universal or local-time basis. It is even quite possible that both kinds of cyclic elements are present, each through correlation with other geophysical factors possessing one or other of these two cyclic characters, *e.g.* atmospheric electrical potential change (uni-

versal time); tides of atmospheric pressure (local time). The existence, however, of exogenous daily cycles has been demonstrated by means of local-time-related, aperiodic phenomena, which are in good measure superimposed on the local-time-related tides of the atmosphere.

It is evident that the solar daily cycle described in this research is clearly of solar-day rather than sidereal-day length. The possible existence of a cycle of the latter period-length was investigated. The existence of one was suggested by the annual cycle involving a positive to negative deviation about 1 AM-to-midnight linear daily trend (Fig. 2), and by the apparent gradual shifting of the solar-day maximum across the day (Fig. 3). This day, being 3.93 minutes shorter than the solar day, would be expected to scan the solar day almost exactly once each year. Similarities in the form of mean-metabolic cycles with cosmic radiation ones (Brown, Webb and Bennett, 1958) and of metabolic cycle amplitude with background radiation cycle amplitude (about half of this radiation is thought to be of cosmic-ray-origin), both give further likelihood for the existence of such a period-length in the living organism. The mean, apparent sidereal-day cycle which was found showed an amplitude of less than 2%. However, in view of the extreme closeness of the period of the year to the length of the cycle of periodic reinforcement of solar-day with sidereal-day cycles, many years of data would obviously be required to resolve the problem of the relative roles of the sidereal day and annual cyclicities in the production of the cycle depicted in Figure 1, B.

It should be emphasized that the low correlations obtained in this study in no manner imply that the correlations with the effective agent would be similarly small. Correlations between consecutive values in parallel cycles of the same frequencies would be expected to be, and are, much higher. The latter, however, could not be used to demonstrate the dependence of one cycle upon another or even on a factor correlated with the second. The cycles of the effective factor may be substantially more regular, and its correlations with organismic metabolism of a much higher order.

Summary

1. Oxygen-consumption was monitored almost continuously in potatoes, *Solanum tuberosum,* in constant conditions, including pressure, for more than two years. A paralleling 8-month study of O_2-consumption in carrots, *Daucus carota,* was also made.

2. The potatoes showed an essentially bimodal mean daily cycle with an average amplitude of the major maximum of 3.7%. The cycles for the two years taken separately were very closely similar.

3. The daily cycle exhibited an annual cycle of form change, with cycles unimodal, inverted, with a 7 PM maximum in February and unimodal with an 11 AM maximum in October. The intervening months yielded bimodal cycles, with graded transitional forms.

4. The daily cycle and its annual fluctuation in the carrot resembled in great detail those obtained concurrently for the potato.

5. An annual cycle in average daily rate of O_2-consumption was found in the potato. The cycle was essentially sinusoidal with minimum in October–November and maximum, the rate about doubled, in April–May.

6. Throughout the two years the 5–6–7 AM deviation in O_2-consumption from linear daily trend was always correlated with the 2–6 AM mean rate of barometric pressure change for the same morning. The sign of this correlation exhibited a characteristic change twice each year, once in the spring and again in the fall.

7. For the two-year period of study the amplitude of the daily cycles showed a linear correlation with the concurrent outside air-temperature, with the sign of the correlation reversing about 57.5° F. With temperature expressed as deviation from 57.5° F., the coefficient of correlation was -0.51 ± 0.049.

8. The data suggest the existence of a rhythmic component of sidereal-day length in the potatoes. Problems in its final resolution are discussed.

9. The evidence points quite conclusively to the possession by the organism, even in so-called "constant conditions," of environmentally imposed oscillations of the natural, daily and annual periods.

10. The fluctuations in the still unidentified, external effective factor appear importantly influenced by, and may possibly even in some measure determine, meteorologic changes of temperature and pressure.

11. The significance of these findings for the problem of the mechanism of the basic daily and annual biological "clock" regulating in "constant conditions" the well-known endogenous organismic rhythms is discussed at some length.

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SULFURIC ACID IN DESMARESTIA

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Several species of *Desmarestia* accumulate acid in their tissues (Blinks, 1951). Kylin (1938) first considered malic acid to be responsible for the low pH values of expressed *Desmarestia* sap. But Wirth and Rigg (1937) and Meeuse (1956) identified the acid as sulfuric. The titration curve of *Desmarestia* sap is that of a strong acid; the pH may be as low as 0.78 and sulfate ions are present in high concentration.

In a review, Blinks (1951) pointed out a correlation between the thickness and relative surface area of three California species of *Desmarestia* and their acid content. The pH of the sap of *Desmarestia munda*, a species with thick blades, is one or less; *D. herbacea* has somewhat narrower and thinner blades and its sap has a pH of two to three; and *D. latifrons*, the most delicately branched of the three species, is only weakly acid with sap of approximately pH five. Blinks also stated that the locality of the acid within the algal tissues is unknown, and suggested that it may occur in the cell vacuoles. He found that the outer cell membranes were injured by acid, and thus it seems unlikely that the acid is free in the cytoplasm.

Kylin (1938) stained *Desmarestia* cells with the vital dye, brilliant cresyl blue, and observed that the vacuoles appeared purple. He concluded that the vacuolar contents were alkaline, because the dye changes from blue to reddish-violet from pH 7.0 to 7.5. He apparently overlooked that fact that the dye also changes from blue to purple from pH 1.0 to pH 0.7. So his conclusion that the vacuoles are alkaline may be questioned. It seems more likely that the vacuoles are strongly acidic. This report provides additional evidence that hydrogen ion accounts for a large part of the vacuolar cation content of the acid-accumulating species of *Desmarestia*.

Methods and Materials

Desmarestia munda and *D. herbacea* were collected at Pebble Beach, California. For comparison, two non acid-accumulating brown algae, *Egregia laevigata* and *Dictyoneurum californicum*, were also collected. The plants were kept in running sea water in the laboratory before use.

The blades were washed free of sea water in 0.6 *M* sucrose for five minutes and extracted with five per cent trichloracetic acid. Sodium and potassium contents of the extracts were determined by flame photometry. Total acidity was determined by titration of hot water-extracts of the algae.

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Amounts of adsorbed cations were assumed to be the same for living and dead tissues, hence the values determined for killed tissues were subtracted from those for living tissues. The difference may represent the intracellular cation content. The apparent osmotic volume of the tissues (Briggs and Robertson, 1957) was estimated as the difference in sucrose apparent free space of living and killed tissues (Eppley and Blinks, 1957). The tissues were killed by boiling or by soaking in fifty per cent ethanol. Ion concentrations are expressed on the basis of the estimated osmotic volumes.

Tissues were placed in a Waring Blendor in 0.8 *M* "tris" buffer (tris hydroxymethyl amino methane-HCl), initial pH approximately 8.5, blended for five minutes, and filtered through cheesecloth for use in methylene blue reduction experiments.

TABLE I

The effects of inhibitors of selective permeability on the rate of acid-loss into the medium of Desmarestia munda tissues as determined by the time required for color change of methyl orange. Tissues in aerated sea water

Expt. 1	Minutes for color change	Expt. 2	Minutes for color change
no inhibitor	>60	no inhibitor	>60
p-chloromercuribenzoate	12	p-chloromercuribenzoate	14
(0.0005 *M*)	16	(0.0005 *M*)	17
NaCN (0.001 *M*)	10		
	12	iodoacetate (0.002 *M*)	2
			1

RESULTS

Several experiments indicate that the acid is within the cells, yet not free in the cytoplasm. Tissue extracts were capable of reducing methylene blue with a variety of substrates, under nitrogen, only if buffered near neutrality. No activity was noted in preparations in which the pH of the extract was less than five or if the tissues were homogenized in unbuffered sea water. Rates of dye reduction were somewhat greater in the presence of ribose and aspartate than with other substrates. Some activity was also present in buffered extracts with no substrate added, but quantitative studies were not made.

In other experiments, discs of the thalli, cut out with a two-cm. cork borer, were tested for acid loss in sea water in the presence and absence of inhibitors. Samples of five or ten discs were placed in ten ml. of sea water containing a drop of methyl orange, and the time required for a color change of the indicator was recorded. The rate of acid loss was much greater in neutralized sea water in the presence of 0.0005 *M* p-chloromercuribenzoate, 0.001 *M* sodium cyanide, and 0.002 *M* iodoacetate than in sea water alone (Table I).

Rates of acid loss in dinitrophenol (0.005 *M*) were determined by measuring the pH of the solution. The curve resulting from a plot of pH against time (Fig. 1) suggests an autocatalytic reaction. This autocatalytic injury is implicit in Blinks' (1951) description of the rates of carotenoid color change in *Desmarestia* as the alga dies.

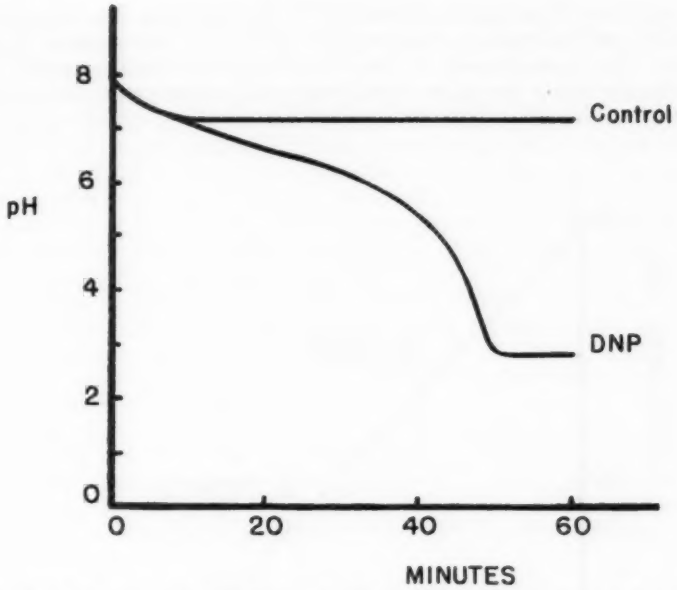

FIGURE 1. Acid release by *Desmarestia munda* tissues in sea water (control) and in sea water containing 0.0005 *M* dinitrophenol (DNP).

On accumulation of the dye, brilliant cresyl blue, the vacuoles of *D. munda* and *D. herbacea* are stained purple in confirmation of Kylin's results (1938). However, we feel the color to be indicative of the change at pH 1.0–0.7, rather than 7.0–7.5.

If the acid is localized within the vacuoles, one might expect the cations normally found in the vacuoles of brown algae to be replaced by hydrogen ions. In *Egregia laevigata* and *Dictyoneurum californicum* (Table II) potassium is the most abundant cellular cation measured. It occurs at a concentration approximately isotonic with sea water. In *D. munda* about 75 per cent of the potassium is replaced by hy-

TABLE II

Potassium, sodium, and hydrogen ion contents of Desmarestia munda, D. herbacea, and two non-acid-accumulating species of brown algae. Values are corrected for the ion contents of killed tissues and represent averages of four determinations. Units milli-equivalents/liter estimated cell osmotic volume

Alga	est. cell. osm. vol.	H	K	Na	Sum
Desmarestia munda	84%	438	148	—	586
Desmarestia herbacea	69%	254	234	13	501
Dictyoneurum californicum	63%	—	523	21	544
Egregia laevigata	71%	—	542	45	587

drogen, and about 50 per cent is replaced in *D. herbacea* (Fig. 2). The reciprocity of potassium and hydrogen ion concentrations agrees with the above mentioned expectation. The approximation of the total cation concentration among the four brown algal species to that of sea water suggests that most of the cation content is accounted for, although magnesium and calcium were not measured and may be present.

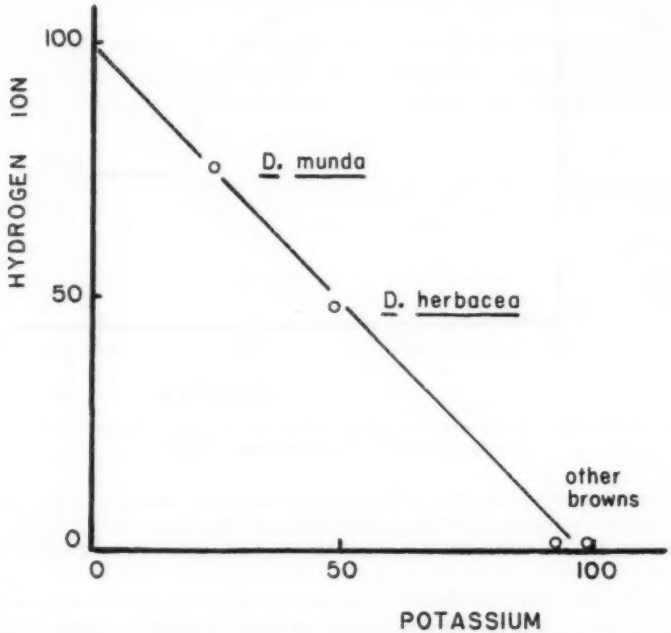

FIGURE 2. Hydrogen and potassium ion contents of *Desmarestia munda*, *D. herbacea*, and two other brown algae: *Egregia laevigata* and *Dictyoneurum californicum*. Units: per cent of total cation content determined.

The binding of large amounts of sodium by dead tissues was detected. This may represent adsorption of the cation to the carboxyl groups of alginic acid, a structural polysaccharide of the brown algae (Wasserman, 1949).

DISCUSSION

The vacuoles of *Desmarestia* contain sulfuric acid in amounts up to 0.44 N, in *D. munda*. Direct evidence for this view is the purple color of brilliant cresyl blue accumulated by the vacuoles of *D. munda* and *D. herbacea*. Indirect supporting evidence includes the following: 1) The acid is lost more rapidly on exposure of tissues to inhibitors which abolish selective membrane permeability than it is in the absence of such inhibitors. In this group are sodium cyanide, iodoacetate, p-chloro-

mercuribenzoate, and dinitrophenol. 2) The autocatalytic release of acid in the presence of dinitrophenol suggests that extra-vacuolar acid injures the cells, causing an increasing rate of acid release. 3) Oxidative metabolism is sensitive to high hydrogen ion concentrations as evidenced by the inability of tissue extracts to reduce methylene blue in unbuffered suspensions. 4) The reciprocity of potassium and hydrogen ion concentrations among the brown algae tested suggests that hydrogen replaces potassium as the most abundant cellular cation in *D. munda*, and that about one-half of the potassium is replaced in *D. herbacea*.

The tonoplasts of *Desmarestia* cells must be quite unique in their resistance to acid injury, and in their permeability characteristics. A hydrogen ion concentration gradient of about 10^7 is apparently maintained between the vacuolar sap and sea water. However, the sea water is probably not the "substrate" for hydrogen ion accumulation. Metabolically produced hydrogen in the cytoplasm may well be the source for vacuolar accumulation. Efforts to leach the acid from the cells so that the progress of acid reaccumulation could be studied have not been successful. The cells are killed as the acid is released.

The production of hydrogen ion due to anaerobic conditions in the interior cells of massive species of *Desmarestia* may explain Blinks' (1951) observation of a correlation between tissue massiveness and acid content. The interior cells of *D. munda* are much larger, contain fewer plastids, and show a greater percentage of purple vacuoles, on staining with brilliant cresyl blue, than the peripheral cells or the cells of *D. herbacea*.

The high acidity of *Desmarestia* cells may limit the vertical distribution of the alga in the intertidal zone. Because injury spreads so rapidly when water circulation is poor, it seems reasonable that the acid-accumulating species must be confined to regions of constant water circulation. *Desmarestia herbacea* occurs below the lowest-lower-low-water tide mark (Doty, 1946) and *D. munda* is limited to the lower portion of the intertidal zone (Smith, 1944).

Summary

1. Brilliant cresyl blue accumulates in the vacuoles of *Desmarestia munda* and *D. herbacea* and the accumulated dye appears purple, indicating that the pH of the vacuolar sap is less than 1.0 or greater than 7.5. However, the expressed saps of these two brown algae have pH 1.0 or less and about 2.0, respectively. The outer cell membranes are injured by the low pH of the sap and methylene blue is not reduced by tissue homogenates at such low pH values.

2. Sodium cyanide, dinitrophenol, iodoacetate, and p-chloromercuribenzoate induce the release of acid from the cells, in which potassium, normally the cation most abundant in brown algal cells, is largely replaced by hydrogen. In *D. munda* hydrogen accounts for 75 per cent of the intracellular cation content. Tissue sodium is largely bound and contributes little to the cellular cation content.

3. The simplest interpretation of these data is that the acid is localized within the vacuoles of *Desmarestia* cells.

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THE SENSITIVITY OF ECHOLOCATION IN THE FRUIT BAT, ROUSETTUS

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Moehres and Kulzer (1956b) have reported that among the Megachiroptera (Old World fruit bats and flying foxes) the genus *Pteropus* orient visually while *Rousettus aegypticus* orient visually but also echolocate. Six additional megachiropteran genera, *Eidolon, Cynopterus, Ptenochirus, Lissonycteris, Eonycteris,* and *Macroglossus,* have all proved, like *Pteropus,* to orient visually and not acoustically. Observations of two additional species of *Rousettus, R. amplexicaudatus* and *R. seminudus* as well as *R. aegypticus,* have confirmed Moehres and Kulzer's conclusions (Novick, 1958). *Rousettus* generate clicks by movements of the tongue and emit these through the open corners of the mouth (Kulzer, 1956) rather than producing sounds laryngeally as do the Microchiroptera (Griffin, 1946, 1952; Novick, 1955; Griffin, 1958).

As far as is known at present all of the Megachiroptera except *Rousettus* are helpless in total darkness. *Rousettus* apparently make use of vision and/or echolocation depending upon the light conditions, the difficulty of their flight path, and the type of flight required (take-offs and landings, for example). The echolocation system used by *Rousettus* has almost surely evolved independently of the system employed by the Microchiroptera. Furthermore, it resembles in design the system serving much the same purpose in the cave-dwelling birds, *Steatornis* and *Collocalia.* The isolation of these three natural sonars in single genera, their simple designs, and their facultative employment (all three genera orient visually in adequate light) make it seem likely that they are recent developments compared with undoubtedly ancient microchiropteran echolocation systems. There is, therefore, considerable interest in comparing the effectiveness of the echolocation system of *Rousettus* in the detection of small objects with that achieved by the Microchiroptera, especially some carefully studied species of the families Vespertilionidae and Phyllostomatidae (Curtis, 1952; Griffin and Novick, 1955; Grinnell and Griffin, 1958).

Since the orientation clicks of *Rousettus, Steatornis,* and *Collocalia* are clearly audible to man, they obviously contain more energy at frequencies below 20 kc than do the orientation pulses of most of the Microchiroptera. The principal component in *Rousettus* clicks is between 12 and 18 kc, depending upon the species and the individual, but overtones and harmonics are present to a considerable degree (Novick, 1958). *Saccopteryx* and *Taphozous* (Emballonuridae) and some species of *Tadarida* (Molossidae) emit partly audible orientation cries. *Rhinopoma* also emit orientation pulses with audible components (Moehres and Kulzer, 1956a). *Rousettus, Steatornis,* and *Collocalia,* though, unlike all of the Microchiroptera,

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produce clicks with relatively little energy above 20 kc. Thus, it appeared that only relatively long wave-lengths would be available for echolocation and that *Rousettus* and the two cave-dwelling birds might be unable to detect obstacles as small as the wires that had been used as standardized test objects for the Microchiroptera (Hahn, 1908; Griffin and Galambos, 1941; Griffin and Novick, 1955; and Grinnell and Griffin, 1958).

A single male *Rousettus aegypticus*, captured in a dimly lighted cave at Eaux Chaudes, Katana, Kivu Province, Belgian Congo in July, 1956, was brought to Harvard University in good health in August, 1956. This bat survived for nine months on a diet of bananas and, after a short period of recuperation from its journey and its restriction to a small cage, flew skillfully in an experimental flight room 32′ long, 12′ wide, and 8′ high. Its ability to avoid a variety of cylindrical test obstacles arranged in a row across the center of this room was tested by methods directly comparable with those previously used to measure obstacle-avoiding skill in the Microchiroptera. This *Rousettus* proved able to avoid surprisingly small wires even in total darkness. Its skill is here compared with that, measured previously, of the vespertilionid, *Myotis l. lucifugus* (Curtis, 1952).

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Methods

After the bat had become accustomed to the problems of flight both in light and in total darkness in the flight room, and to the task of dodging between vertical obstacles suspended from the ceiling across the middle of the room, we tested its ability to avoid cylindrical obstacles, spaced 53 cm. apart, varying in size from cardboard tubes 5 cm. in diameter to bare metal wires 0.28 mm. in diameter. In each case these obstacles were suspended in a movable frame in a plane parallel to the end walls of the room. This plane had to be crossed by the bat in flying from its roost at one end to its roost at the other end. We forced such flights by agitating the roost which was a loosely suspended horizontal bar of wood. The bat would take off and fly the length of the room to the opposite roost or would, on occasion, make several flights back and forth before landing. In each of the tests considered below, the frame holding the obstacles was shifted horizontally in the dark just before each flight so that the absolute position of the obstacles and their location relative to the walls were unknown to the bat, though their position relative to one another was constant. Thus, the bat could not learn the location of the open spaces nor could it depend on following the walls because the space adjacent to the walls was frequently and randomly too narrow to permit passage. The room was totally dark during all these observations, but we often noticed by listening to the bat's audible clicks or to its wingbeats that it hesitated in front of the obstacles and executed dodging maneuvers to pass between them.

The *Rousettus* was thus required to fly through an obstacle plane and its accuracy of echolocation was evaluated in terms of its ability to avoid the obstacles. One must consider whether it was constantly and equally motivated to avoid collisions and whether its physical agility was sufficient for it to make the maximum use of its orientation system. The flights were scored simply as hits or misses by means of the sound of hits or in doubtful cases by inspecting the obstacles in light switched on immediately after the bat's passage. A hit always caused a clearly visible, sustained vibration of the obstacles as they were suspended from rubber bands. All hits were considered equal although some undoubtedly represented the bat's

TABLE I

Comparison of the obstacle avoidance scores of a Rousettus aegypticus with those of Myotis l. lucifugus (Curtis, 1952). The wires or other cylindrical obstacles were arranged vertically and spaced 53 cm. apart for Rousettus and 30 cm. apart for Myotis

Diameter of obstacle (mm.)		*Myotis l. lucifugus*		*Rousettus aegypticus*	
		No. trials	% misses	No. trials	% misses
Cardboard tubes	25	—	—	109	76%
Rubber tubing	19	—	—	161	78%
Rubber tubing	13	—	—	100	77%
Rubber tubing	6	—	—	50	80%
Metal rods	4.76	140	85%	—	—
Insulated metal wires	3	—	—	442	85%
Bare metal wire	1.5	—	—	200	77%
Bare metal wire	1.21	3820	82%	—	—
Bare metal wire	1.07	—	—	280	68%
Bare metal wire	0.68	480	77%	—	—
Bare metal wire	0.65	—	—	225	58%
Bare metal wire	0.46	—	—	134	45%
Bare metal wire	0.35	660	72%	—	—
Bare metal wire	0.28	—	—	50	18%
Bare metal wire	0.26	600	52%	—	—
Bare metal wire	0.12	530	38%	—	—
Bare metal wire	0.07	460	36%	—	—

inability to maneuver successfully even though it had detected the obstacle, and some represented light touches by the wingtips which may have been sufficiently painless to call for no great effort to avoid their occurrence. Unlike the Microchiroptera, this *Rousettus* rarely turned back from the obstacles. Its position and attitude in passing through the obstacle plane were recorded on about 40 flights with a camera and electronic flash. All wing positions from completely spread to considerably folded were photographed both just before and just after passage through the barrier, but we could not determine whether the bat was reducing its potential collision diameter just at the obstacle plane. Its maximum wingspread was about 75 cm., and while we cannot accurately estimate its mean wingspread this must have been at least 45 cm. or very little below the spacing between the wires.

Finally, the possibility that the bat would detect the presence of the obstacles by their fastenings to the ceiling and/or floor and learn that they were suspended ver-

tically between these two points was excluded by framing the obstacle plane with uniform fiberboard so that only the obstacles themselves and not their fastenings were exposed to acoustic or visual inspection. As a last precaution, lest the bat learn to recognize the position of the obstacles by listening to the movement of the frame between flights, the readjustment was covered with loud noise. The nature and size of the obstacles used are shown in Table I.

TABLE II

Experiments with a captive Rousettus exposed to thermal noise while flying through a row of vertical wires, 3 mm. in diameter spaced 53 cm. apart. All flights in total darkness except as noted. The noise was filtered with high pass (HP) or low pass (LP) electronic filters as noted

Date	Conditions of test	No. misses/No. trials	Per cent misses	Remarks
Apr. 23	Quiet	30/40	75%	
	Noise, 25 kc HP	0/10	0	Totally disoriented
	Quiet	17/20	85%	
	Noise, 25 kc HP with lights on	9/10	90%	Flew normally
	Noise, 15 kc LP	4/10	40%	Somewhat disoriented
	Quiet	7/10	70%	
	Noise, 15 kc LP	3/10	30%	Disoriented, but less so than at 25 kc HP
	Quiet	10/10	100%	
Apr. 26	Quiet	10/10	100%	Reluctant to fly
	Noise, 25 kc HP	0/8	0	Badly disoriented
	Quiet	6/6	100%	Very tired
Apr. 28	Quiet	8/10	80%	
	Noise, 15 kc LP	1/10	10%	Badly disoriented
	Quiet	4/10	40%	Tired
May 3	Died			
Averages of all tests	Quiet	93/116	79%	
	Noise, 25 kc HP	0/18	0	
	Noise, 25 kc HP with lights on	9/10	90%	
	Noise, 15 kc LP	8/30	27%	

RESULTS

The results are presented in tabular form. The only data excluded from consideration are those which were obtained when the bat was clearly fatigued or in poor condition near the end of a long series of trials or after many days of inactivity. The data are compared directly in Table I with similar data obtained by Curtis (1952) with *Myotis l. lucifugus.*

A short series of experiments was carried out to compare the resistance of *Rousettus* to interference with its echolocation by thermal noise but before further studies could be completed the bat died, possibly of injuries sustained in these experimental flights. The data are shown in Table II, because they indicate a mark-

edly greater vulnerability to interference by noise than occurs with the Vespertilionidae (Griffin, 1958). Thermal noise was generated in 20 electrostatic loudspeakers adjacent to the plane of obstacles. This noise was limited in frequency band, by electronic filters, in one of two ways. Either the filter was set at 15 kc high pass so that frequencies above 15 kc were generated at a high level while lower frequencies were attenuated progressively at 24 db per octave, or else a 25 kc low pass filter was used to transmit audio frequencies while attenuating ultrasonic components of the noise, also at 24 db per octave. Without noise, the bat avoided 3 mm. wires 79% of the time in the dark. In the light, and with the noise, in a very short series, it avoided the wires 90% of the time. But in the dark the bat was incapable of avoiding these wires at all in intense noise above 25 kc. In noise below 15 kc, it scored 27% misses. The bat's total inability to avoid large wires in noise above 25 kc and its very poor performance in noise below 15 kc suggest several hypotheses. If we assume that the poor performance was due to unfavorable signal-noise ratio at the same frequencies, then we have evidence that *Rousettus* depends upon a wide range (from less than 15 kc to more than 25 kc) of frequencies in echolocation. But alternatively the analytical ability of *Rousettus*' ears may not suffice for distinguishing a 14 kc echo from either type of noise tested, that is, we may simply have shown that the accuracy of acoustic orientation in *Rousettus* can be reduced (even totally) by noise. The results may also have been complicated by the bat's panic, discomfort, loss of motivation, or confusion in an unusual situation aside from its ability to perceive echoes in a noisy environment.

Discussion

In these experiments, the wires were less widely spaced relative to the wingspread of *Rousettus* than in Curtis' experiments with *Myotis*, but *Rousettus* almost always approached the plane of the obstacles perpendicularly while *Myotis* often approached obliquely. Our flight room was also considerably larger than the $15' \times 9' \times 6'$ room used by Curtis. The percentage of misses for relatively large obstacles was, nevertheless, almost exactly the same—85.0% for *Myotis* with 4.76-mm. rods and 84.5% for *Rousettus* dodging 3-mm. wires. *Rousettus* was slightly less successful at avoiding even larger obstacles (cardboard and rubber tubes) but these tests were conducted early in the bat's experience in the exacting task of flying in a dark room (with its multiplicity of echoing surfaces).

This *Rousettus* was able to detect and avoid, with a considerable degree of success, wires as small as 1.07 mm. in diameter. Only when confronted with wires of less than 1 mm. did its skill fall seriously below its own standards as well as those of *Myotis*. *Rousettus*' score decreased rather gradually. If we consider its poor performance (18% misses) against 0.28-mm. wires as due to chance, then *Rousettus* was clearly detecting 0.46-mm. wires against which it scored 45% misses. Even 18% misses against 0.28-mm. wires may have represented some degree of echolocation for, when flying in a noise field, this bat did even more poorly (100% hits) against 3-mm. wires. It seems reasonable that the ease with which a small object is echolocated depends upon its position relative to the angle of sound emission and its beaming and the angle of sound reception. Thus there is likely to be an optimal angle of approach (probably, but not necessarily, straight ahead) where the maximum echo will be received and less easily detected obstacles will be echo-

located. Obstacles which lie less optimally relative to the bat will have to have more effectively echoing surfaces to be detectable. Thus the bat might well succeed in avoiding a 0.46-mm. or 0.65-mm. wire only if it chanced to approach it favorably and so its score when working against obstacles of marginal size would be an average of chance misses, active misses, and "blind" hits. One of the limiting factors in exploring the threshold of echolocation is the danger of serious injury to the bat every time it collides with an obstacle. Such collisions may be major accidents or simply touches. Collisions with small wires tend to be more serious than those with large obstacles. *Rousettus'* performance varied considerably from trial to trial. Whenever possible we ran long series of tests and interspersed tests with 3-mm. wire between those with smaller sizes. The results were consistent with the average scores listed in Table I.

The design of *Myotis* orientation pulses is very different from that of *Rousettus* clicks. *Myotis* pulses are produced laryngeally and emitted through the open mouth. They have a frequency modulated pattern with a gradually falling frequency starting on the average at about 80 kc and ending at about 40 kc but with beginnings ranging from at least 60 to 120 kc. Similar variety among terminal frequencies also occurs. Thus *Myotis* in single pulses and in consecutive pulses produce prominent frequencies covering about two octaves (Griffin, 1958; Novick, 1955). Furthermore, harmonics also occur in *Myotis* pulses and represent a second octave sweep within the pulses in which they occur. The importance of the harmonics as components of the outgoing pulses and the returning echoes and in the carrying of information about the environment to the bats has not been evaluated. In *Rousettus*, the pulses are produced by tongue clicks and are impure in frequency and irregular in frequency pattern. The bulk of the energy, however, appears to be in the range of about 12 to 18 kc. Additional energy is scattered from 6.5 to over 100 kc with a second maximum at about 20 to 40 kc (Moehres and Kulzer, 1956a; Kulzer, 1956; Novick, 1958).

Summary

1. The ability of a single specimen of the fruit bat, *Rousettus aegypticus*, to avoid test obstacles of various sizes by echolocation in total darkness was tested. This bat avoided vertically placed 3-mm. metallic wires 85% of the time. Its success declined gradually as the wires were reduced in size but the bat displayed considerable success (68% misses) against 1.07-mm. wire and did significantly better than chance (45% misses) against wires 0.46 mm. in diameter.

2. These results have been compared with those of Curtis (1952) who studied the vespertilionid, *Myotis l. lucifugus*.

3. *Rousettus'* success at echolocation was considerably reduced when it was forced to fly in a field of intense thermal noise.

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ELECTROPHYSIOLOGICAL STUDIES OF ARTHROPOD CHEMORECEPTION. III. CHEMORECEPTORS OF TERRESTRIAL AND FRESH-WATER ARTHROPODS[1]

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While an extensive literature documents the role of chemoreceptors in the behavior of invertebrates (Hodgson, 1955), the small size of chemoreceptor cells is a major handicap in any attempt to study their functions using conventional electrophysiological procedures (Chapman and Craig, 1953; Roys, 1954). Barber (1956) recorded afferent impulses from neurons which supply the gnathobase chemoreceptors of *Limulus* and noted an increase in nerve activity when aqueous extracts of marine bivalves were applied to the gnathobase. Use of microelectrodes enabled Schneider (1957) to record afferent impulses from groups of antennal chemoreceptors in male silkmoths (*Bombyx*) during stimulation with extracts of the scent glands from female moths. Possible synaptic effects between receptor cells and nerves supplying them, or the unpredictable numbers of cells represented in most recordings, make it difficult, however, to interpret the results in terms of single unit activity of the actual chemoreceptor cells.

A relatively simpler technique is that of recording the afferent impulses from primary chemoreceptor cells through the same fluid which is applied as a stimulus (Hodgson, Lettvin and Roeder, 1955). This method has thus far been applied only in studying contact chemoreceptors of two animals: labellar chemoreceptors of the blowfly *Phormia* (Hodgson and Roeder, 1956; Wolbarsht, 1957) and tarsal chemoreceptors of the butterfly *Vanessa* (Morita *et al.*, 1957). The conclusions from studies of these two preparations point to a number of unexpected properties of primary chemoreceptor cells.

With both *Phormia* and *Vanessa*, it was found that different chemoreceptor cells were specialized to respond, not to the different modalities of stimuli generally held to be effective for contact chemoreceptors of vertebrates (*e.g.* Beidler, 1952), but either to sugars or to various non-sugars, with the presence of a water-specific receptor also strongly indicated in *Vanessa* (Morita *et al.*, 1957). Seemingly at variance with the usual concept of single specificities of receptor cells (Granit, 1955), a single primary receptor cell of *Phormia* may respond to chemical, tactile, and thermal stimuli within normal physiological ranges (Hodgson and Roeder, 1956). Unfortunately, information on this point is not available for *Vanessa*.

In view of these unexpected results, and the lack of any comparable electrophysiological data on primary chemoreceptors of other invertebrates, it seemed desirable that the method of recording through fluid-filled, externally applied electrodes

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should be tried on chemoreceptors of a wider variety of animals, in order to determine how generally the characteristics found in *Phormia* and *Vanessa* receptors may apply to the functions of other primary chemoreceptor cells. For technical reasons, this method is best adapted to recording from chemoreceptors in arthropods (Hodgson, Lettvin and Roeder, 1955). The object of the present paper is to report the results of tests conducted using this method upon the chemoreceptors of some terrestrial and fresh-water arthropods. In each case where the method could be successfully applied, answers to the following questions were sought: (1) Does the same receptor cell respond to chemical, tactile, and thermal stimuli within normal physiological ranges? (2) What modalities of chemical stimuli excite the individual primary chemoreceptor cells? (3) Does the relationship between the reaction of the animal to chemicals and the range of sensitivity of its chemoreceptors indicate a peripheral discrimination mechanism, such as found in *Phormia*?

Methods

Thirty-seven species, representing the major classes of arthropods and eight orders of insects, were tested. These species are arranged according to taxonomic status below. All specimens were collected in the field and tested within 12 hours after capture. The animals were allowed to drink water to repletion, but no attempt was made to control their diet prior to testing. At least three individuals, usually more, belonging to each species were studied.

The technique of recording action potentials from chemoreceptors using externally applied, fluid-filled electrodes has been described in detail elsewhere (Hodgson, Lettvin and Roeder, 1955; Hodgson and Roeder, 1956). This technique was used with only such minor modifications as were necessary to manipulate the variety of receptor-bearing appendages tested. All experiments were tape recorded and photographs made from the tape recordings, beginning one-half second after the stimulus was applied, thus avoiding the base-line fluctuations which commonly accompany the stimulus artifact.

The species tested were as follows, with each group and each species yielding potentials from chemoreceptors designated by an asterisk. (Except as otherwise noted, identifications were checked through the courtesy of Dr. R. E. Blackwelder of the U. S. National Museum.) *Class:* Crustacea*—Cambarus bartonii sciotensis* (Det. H. H. Hobbs, Jr.); *Class: Arachnida*—Latrodectus mactans (black widow spider), Theridion tepidariorum (house spider); *Class: Diplopoda**—Pseudotremis sp. (Det. H. F. Loomis), Pseudopolydesmus serratus* (Det. M. Walton); *Class: Insecta; Order: Odonata*—Aeschna constricta, Libellula pulchella, Progomphus obscurus; *Order: Orthoptera**—Acheta assimilis (common field cricket), Ceuthophilus gracilipes* (cave cricket), Crytocercus punctulatus (wood-eating roach) (Det. L. R. Cleveland), Hadenoecus putaneus* (cave cricket), Scudderia furcata (katydid); *Order: Hemiptera*—Arilus cristatus, Oncopeltus fasciatus (large milkweed bug); *Order: Coleoptera*—Cicindela sexguttata (six-spotted tiger beetle), Dineutes americanus (whirligig beetle), Dytiscus fasciventris (large diving beetle), Laccophilus maculosus (common pond beetle), Nicrophorus tomentosus (carrion-beetle), Phymatodes dimidiatus (longhorn beetle), Saperda candida (apple tree borer), Silpha americana (carrion beetle), Tropisternus lateralis (keeled water beetle); *Order: Megaloptera*—Carydalus cornutus (dobsonfly); *Order: Neurop-*

tera—Chrysopa sp. (golden eyed lacewing); *Order: Diptera**—Amoebaleria defessa* (cave fly) (Det. C. H. Curron), Tipula trivittata (crane fly); *Order: Lepidoptera**—Atlides halesus (purple hairstreak); Epargyreus clarus* (silver spotted skipper), Limenitis arthemis astyanax* (red spotted purple), Papilio marcellus* (zebra swallowtail), Papilio philenor* (pipe vine swallowtail), Protoparce quinquemaculata (five-spotted hawk moth), Speyeria cybele* (great spangled britillary), Tropaea luna (luna moth), Vanessa atalanta* (red admiral).

The chemicals tested were sodium chloride, sucrose, d-levulose, glycine, DL glutamic acid, citric acid, oil of citronella and oil of wintergreen. Sodium chloride was tested as a 0.25 molar aqueous solution. Oils of citronella and wintergreen were tested by bringing swabs soaked in these chemicals to within an inch of the sensory structure. Although quantitative control of stimulus concentration was not obtained by this method, the results obtained with these two oils were quite reproducible. All of the other chemicals were mixed with sodium chloride so that the final test solution was an unbuffered aqueous solution containing 0.1 molar NaCl and a 0.25 molar concentration of the test chemical. Results were compared with activity recorded when 0.1 molar NaCl was applied alone.

Temperatures were measured with a thermistor implanted just under the cuticle near the receptor being studied. The temperature was changed by bringing a warm glass rod or small ice-pack near the preparation. Spike potentials from mechanoreceptors were recorded by bending sensilla or whole appendages with needles. Certain departures from the usual tests are described at appropriate points below.

Results

All of the preparations yielded numerous spike potentials originating from tactile receptors, thus providing assurance that the preparations were alive when studied. In only five orders of the arthropods tested, however, was it possible to obtain unequivocal recordings from chemoreceptors. These five groups are designated by asterisks above. The several factors believed to be responsible for failure to record action potentials in all of the tested species are considered in the discussion, and a complete description of the results will be presented only for those forms in which chemoreceptors could be studied using fluid-filled electrodes.

1. DECAPODA *Cambarus bartonii sciotensis* (16 individuals)

This large crayfish proved to be an exceptionally interesting experimental animal. Recordings could be made with the usual 0.1 molar NaCl conducting solution in the electrode, or else by using distilled water or pond water as a solvent for the chemicals. Although the results showed few differences whichever solvent was used, all of the tests were run with chemicals dissolved in distilled water, thus avoiding any possible complications of the sodium chloride.

The antennae and the lateral branches of the antennules were alike in yielding only records of mechanoreceptors at low amplitudes (30 μV). From the entire medial branch of the antennule, however, it was possible to record a variety of spike potentials ranging in amplitude from 30 μV to 500 μV. The large-amplitude spikes (200 μV to 500 μV) were recorded only when the antennule was bent. Consequently, the cells giving rise to these potentials, which are relatively few in this

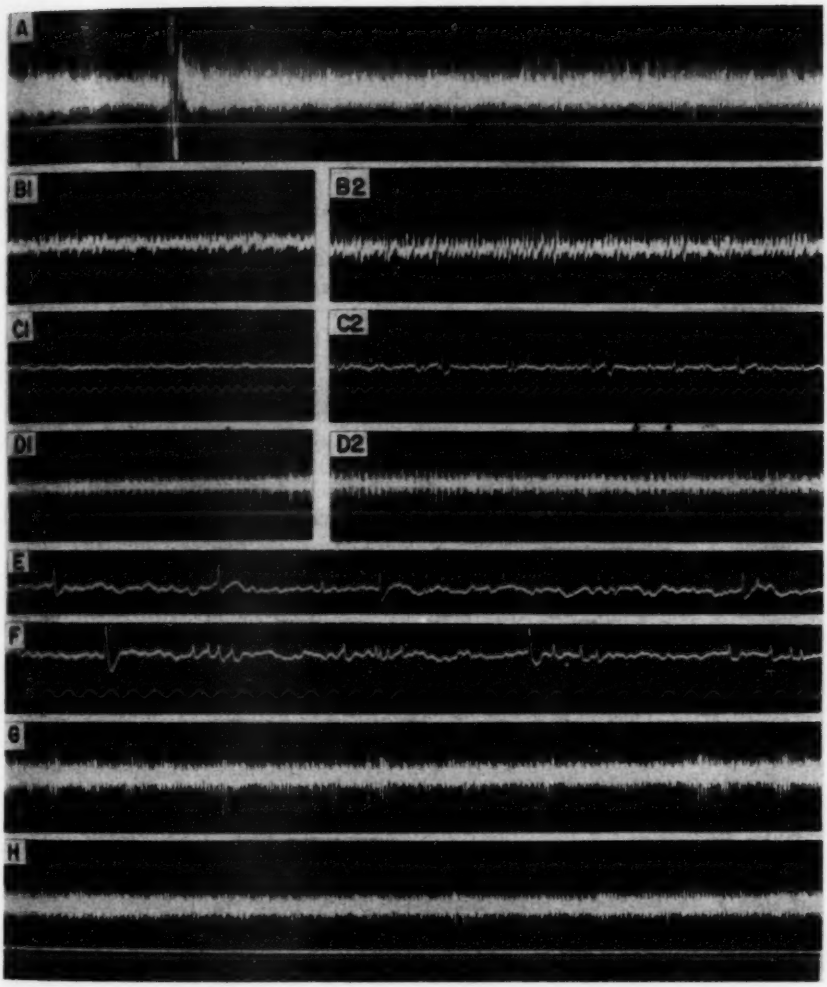

FIGURE 1. Typical spike potentials from arthropod chemoreceptors. *A*, response of medial branch of *Cambarus* antennule to glutamic acid; *B1*, single sensillum on *Cambarus* walking leg, tested with distilled water; *B2*, same as *B1*, except glycine test solution; *C1*, *Pseudopolydesmus* tarsus, NaCl control; *C2*, same as *C1*, except sucrose test solution; *D1*, spontaneous activity, *Hadenoecus* tibia; *D2*, same as *D1*, except exposed to citronella vapor; *E*, single tarsal sensillum of *Epargyreus*, control NaCl solution; *F*, same as *E*, except sucrose test solution; *G*, antenna of *Amoebaleria*, distilled water in electrode; *H*, same as *G*, except exposure of antenna to oil of citronella vapor. Time bases for all records, 100 cycles per second. Consult text for additional details.

branch of the antennule, are mechanoreceptors. The majority of the spikes have amplitudes of 30 μV to 50 μV. These respond to the application of glycine and glutamic acid, of the test series of chemicals used. Because a number of different amplitudes of spikes were recorded even with the smallest practicable areas of electrode contact, it was not possible to determine whether identical cells were responding to both chemical and tactile stimuli. Record A of Figure 1 is taken from an experiment in which a test solution of glutamic acid was allowed to flow around the medial branch of an antennule without changing electrode contact. Activity recorded when the antennule is in distilled water (on the left of the large stimulus artifact) is negligible, but many small-amplitude spike potentials follow the introduction of the glutamic acid. The frequency of firing during chemical stimulation was not influenced by temperature changes within the range tested—five degrees (C.) above or below the room temperature of 25 degrees.

Chemoreceptors were also found on the first two pairs of walking legs. The chemoreceptors were located on the chelae and, to a lesser extent, elsewhere on the protopodites of those legs. The external chemosensory structures can be recognized in *C. bartonii* as tufts of setae, numbering ten to twenty setae per tuft, each such tuft arising from a circular depression in the cuticle. Contact of the electrodes with other parts of the cuticle failed to terminate the open circuit condition between the indifferent electrode inside and the recording electrode outside the cuticle. The best records were obtained after the claw had been allowed to dry at room temperature for thirty minutes following its removal. This prevented short circuits between the recording and indifferent electrodes. By teasing apart the hairs of a single tuft, the tip of an electrode could then be positioned over a single sensory hair. In this way the firing of a single chemoreceptor cell could be studied. The spike potentials recorded from different sensilla ranged from 30 to 60 μV in amplitude. It was found that these receptors resemble chemoreceptors on the antennule in being insensitive to test chemicals other than amino acids of the series used. (Records B1 and B2 of Figure 1 illustrate typical results during applications of a control NaCl solution, and the test mixture of NaCl and glycine, respectively.) The chemoreceptors on the first two walking legs were never observed to respond to mechanical movement of the sensory hairs during recordings. The small size of the hairs (about 20 microns in length) and their position surrounded by rigid cuticle would appear to render their usefulness as tactile receptors unlikely. The insensitivity of these receptors to temperature changes within the range tested resembles that of the receptors on the antennule. Impulses from chemoreceptors were not detected from the chelipeds, third maxillae, or elsewhere on the body of the crayfish using the present method.

Behavioral experiments were run to check the possibility of a peripheral discrimination for amino acids. Ablations of antennae, antennules, or the first two pairs of walking legs, and combinations of these operations, were performed on thirty crayfish. The results were difficult to interpret in many cases because of abnormal behavior of operated animals. It was easy to demonstate, however, that the animals can locate food using the first two pairs of walking legs, even when antennae, antennules, and maxillipeds are removed. Activity resembles that during normal feeding and can be initiated by injecting 0.25 molar solutions of glycine and glutamic acid into the water, while even intact animals fail to give clear-cut responses to the other test solutions. Thus there seems to be a clear correlation between the elec-

trophysiological data and the behavioral results. Attempts to determine by behavioral tests whether the antennae and lateral branches of the antennules bear chemoreceptors yielded results which could not be unequivocally interpreted. Doflein (1910), on the basis of behavioral tests, has reported that the antennules of decapods contained chemoreceptors, and Luther (1930), using similar methods, reported chemoreceptors on mouth parts, walking legs, and pincers of brachyurans.

2. DIPLOPODA *Pseudotremis* sp. (4 individuals); *Pseudopolydesmus serratus* (4 individuals)

In both *Pseudotremis* and *Pseudopolydesmus* many action potentials could be recorded from the tips of the antennae and from the tips of the legs when an electrode filled with 0.1 molar NaCl was applied to those parts. In *Pseudotremis,* the smaller species, the action potentials were never more than 40 μV in amplitude, and all clearly responded to mechanical bending of the antenna or leg. In *Pseudopolydesmus* the largest spikes from the antenna were about 60 μV in amplitude, and those from the tarsus were about 80 μV. All of the larger spikes increased in frequency during bending of the appendages being tested, and it was therefore assumed that these spikes represented the afferent impulses from mechanoreceptors. Spike potentials of smaller amplitude (30–50 μV) from tarsi of *Pseudopolydesmus* occurred with increased frequency when the tarsi were bent, or sugars applied. (See Fig. 1C.) They did not change during application of other test solutions or during temperature changes within five degrees (C.) of the room temperature of 25 degrees. No significant changes in the frequency or pattern of impulses were noted in recordings from the antennae of the two species when chemical stimuli were applied.

The small trichoid sensilla which probably enclose the actual chemosensory cells on the tarsi of *Pseudopolydesmus* are too closely spaced to make possible a restriction of the area of electrode contact to a single sensillum. Attempts to record activity using electrodes filled with distilled water were likewise unsuccessful. In view of the smaller size of the mechanoreceptor spikes recorded from *Pseudotremis,* and the generally smaller size of action potentials from chemoreceptors as compared with mechanoreceptors, it would hardly be expected that chemoreceptor spikes from *Pseudotremis* would be detectable above the inherent "noise level" of the apparatus. Behavioral test showed that sucrose or levulose, placed in contact with the tarsi, initiated feeding responses even after the antennae were removed. Tarsal contact with citric acid caused the animals to move away from the test solution, but this was the only test solution, other than the sugars, which elicited a behavioral response. With the exception of citric acid, receptors for which could not be detected electrophysiologically, the behavioral and electrophysiological results suggest the existence of a peripheral discrimination mechanism.

3. ORTHOPTERA *Ceuthophilus gracilipes* (7 individuals); *Hadenoecus putaneus* (3 individuals)

The orthopterans tested showed considerable variation, some of which appears to be related to habitat. *Cryptocercus,* a wood-eating roach, was completely refractory to the recording method, except for a few mechanoreceptors in the antennae and palpi. A larger number of tactile receptors were recorded from the antennae and

palpi of the katydid, *Scudderia*, and the field cricket, *Acheta*. Best results, however, were obtained with the cave crickets *Ceuthophilus* and *Hadenoecus*, which have antennae elongated to many times the length of the body and also have unusually long legs and palpi. The data support the generally expressed assumption that these anatomical modifications are associated with hypertrophy of tactile and chemical senses which would presumably be of selective value in dark subterranean environments.

In tests of seven adult specimens of *Ceuthophilus* and three of *Hadenoecus*, the antennae were found to contain spontaneously active and quick-adapting mechanoreceptors (spike amplitudes 50–80 μV) along with spontaneously active, relatively non-adapting chemoreceptors (spike amplitude 20–40 μV). The latter were seen in one antennal preparation of *Ceuthophilus* and all three preparations of *Hadenoecus*. The frequency of the small spikes did not change during application of any of the test chemicals in solution, or during temperature changes between 20 and 30 degrees C., but did increase when swabs soaked in citronella or wintergreen were brought near the region of the antenna in contact with the electrode. Essentially similar results were obtained from recordings of the receptor activity in both the maxillary and labial palpi and the tarsi of *Ceuthophilus* and *Hadenoecus*. In addition, small spikes (30–50 μV) were recorded from the trochanter and tibia of the prothoracic and mesothoracic legs of *Hadenoecus*, in six out of eight preparations when the legs were exposed to vapors of wintergreen or citronella. Mechanical bending of sensilla on the trochanter and tibia also increased the frequency of these same spike potentials. Record D1 of Figure 1 shows the spontaneous activity of receptors in the tibia of a prothoracic leg of *Hadenoecus*, and record D2 shows the increase in frequency of spikes during application of citronella vapor. No effects of the test chemicals in solution could be detected in either *Ceuthophilus* or *Hadenoecus*, and chemoreceptor activity could not be recorded from the cerci, ovipositor, general body surface, or the larger spines on the legs of either species. *Ceuthophilus* did not give any clear-cut behavioral response to citronella or wintergreen in tests of the intact animals, but *Hadenoecus* gave intense avoidance reactions, moving quickly away from these stimuli. Removal of the antennae and palpi did not abolish this reaction in *Hadenoecus*, which always responded most strongly when stimuli were near the legs.

4. LEPIDOPTERA

Nine species of Lepidoptera were tested. Only a few impulses associated with tactile stimulation could be recorded from the antennae of any of these species, even when vapors were applied. In all six species of butterflies tested, records were obtained from the tarsal receptors (described by Minnich, 1921). Tests upon the tarsal sensilla of *Epargyreus* and *Limenitis* revealed that each sensillum had a few receptor systems functioning similarly to that in the labellar hairs of *Phormia*. (Compare the records E and F of Figure 1, taken from tests of a single tarsal sensillum of *Epargyreus*, and note that the small spike potentials predominate only in record F when sugar is present in the electrode.) The maximum number of receptors represented in recordings from single sensilla of these two species is four, and the minimum two. Variations within these limits were commonly encountered in comparisons of the records from several hairs, even on the same tarsus. The

variations characteristically occurred in the smaller spike potentials, but under the conditions of these tests all of the smaller spikes increased in frequency during stimulation with sugars, and the largest spike responded with increased frequencies during application of any of the non-sugar solutions. These receptors were not observed to respond to vapors of citronella or wintergreen.

With the other species of butterflies tested, there appeared to be as many as 12 different receptors associated with each tarsal sensillum and the records were too complex for analysis of the functions of any single receptor cells. Responses to tactile stimulation were obtained in tests with tarsal hairs of all the butterflies used; in those preparations involving only a few fibers it was clear that all fibers responded to bending of the tarsal hair, and probably this was the case with the many-fiber preparations also, but this could not be determined with certainty because of the complexity of the records. The frequency of impulses recorded during continuous stimulation of single sensory hairs of *Epargyreus* and *Limenitis* was increased by temperature rises of as little as 1.2 degrees C. These particular tarsal receptors, then, bear a greater resemblance to the labellar chemoreceptors of flies than do any of the other preparations (excluding the labellar chemoreceptors of *Amoebaleria*) encountered in this survey. Feeding responses (proboscis extensions) in butterflies are known to be elicited by sugars, with negative responses being elicited by other types of chemicals (Dethier, 1953; Minnich, 1921). A peripheral mechanism for discrimination of acceptable and unacceptable chemicals is thus indicated by both the behavioral and electrophysiological results with butterflies.

Tarsal chemoreceptors were not detected in any of the three species of moths. No impulses could be recorded from the trichoid sensilla described by Frings and Frings (1949) on the proboscis of lepidopterans. The characteristics of the records obtained from such tests indicated, however, that a short-circuit between the recording and indifferent electrodes, established through the fluids in the proboscis, probably accounted for the lack of any spike potentials detected through an active electrode near the tip of the proboscis.

5. DIPTERA *Amoebaleria defessa* (7 individuals); *Tipula trivittata* (3 individuals)

Studies on four genera of Diptera having been previously reported (Hodgson and Roeder, 1956), the present work was confined to two types in which the chemoreceptors might be expected to be of special interest. The helomyzid fly *Amoebaleria* was tested because of its occurrence in caves, a habitat often associated with hypertrophy of chemical or tactile senses (Hodgson, 1955), and the crane fly *Tipula* was tested because the branching structure of its antennae suggested that recordings might be made from one or a few antennal receptors in a single antennal branch. Only *Amoebaleria* yielded results of interest, however.

The labellar chemoreceptors and chemoreceptors within the tarsal hairs of *Amoebaleria* proved to function similarly to those in *Phormia*, in that they exhibited *L* and *S* spikes when stimulated by sugars or non-sugars, and showed comparable responses to tactile and temperature stimulation. Some data on olfactory receptors were obtained in recordings from the antennae of *Amoebaleria*. A typical result, obtained by placing a fluid-filled electrode on the antenna, is shown in record G of Figure 1. Distilled water is adequate in the electrode, and the results are essentially

the same whether contact is made with the distal tip of the antenna or the enlarged third segment near the base of the antenna. Ablation experiments show that most of the activity recorded originates in the third segment of the antenna in either case. The abundant spikes which seem to represent the basal level of receptor activity in the absence of externally applied stimulation are not affected by any of the test solutions applied, but are decreased in frequency by vapors of wintergreen, or citronella (see record H of Figure 1). This result was so contrary to anticipated findings that tests were run with benzene, toluene, and carbon tetrachloride vapors, all of which produced similar reversible decreases in amount of receptor activity. Unfortunately, so little is known of the natural history of this fly that it is impossible to say what might constitute the normal olfactory stimuli.

Tactile effects upon the antennal receptors were observed only when the surface of the antenna was prodded or bent in excess of any amount of stimulation which the antenna would encounter in flight. Blowing upon the antenna during a recording or varying the temperature from 20 to 28 degrees C. produced no discernible effect upon the frequency or pattern of the impulses recorded. Attempts to make similar antennal recordings using other species of flies have yielded only negative results.

Discussion

In view of the considerable differences in chemoreceptors which have already been reported from electrophysiological studies of mammals (Beidler, Fishman and Hardiman, 1955) it is not surprising that much greater differences should be found among members of such a heterogeneous group as the arthropods. It seems clear that sensitivities to tactile and temperature stimuli within the normal physiological range are not essential characteristics of primary chemoreceptor cells, even among the arthropods, because several exceptions to this situation were found as soon as tests were made of chemoreceptors other than those on the fly labellum. Yet it would probably be incorrect to regard the labellar receptors as primitive or unspecialized receptor cells. Their similarity to receptors in the tarsal sensilla of at least two of the butterflies tested suggests that a sensitivity of the same cell to more than one type of energy in the environment may have a high selective value in cases where only a relatively small number of receptors contact a substrate, many features of which are significant for the animal's behavior. This certainly would be the case with receptors on the tarsus or proboscis of a fly or butterfly, or on the tips of the tarsi of a millipede. The demonstrated multiple sensitivities of single receptor cells in those locations may, therefore, be one of the solutions which evolution has produced for the problem of obtaining a variety of information about the environment when only very small areas of the body are actually in contact with the environment. Whether the several types of stimuli all eventually affect the same excitatory process within a single receptor cell will have to be determined by further investigations. Cases of double specificities of receptors in vertebrates, such as the temperature-touch receptors of the rattlesnake facial pit (Bullock and Diecke, 1956), have been reported but it is very doubtful that more than one type of stimulation *normally* acts upon the same receptor units, and even if this were true these would have to be considered exceptions to the general rule of single specificities for single receptors (Granit, 1955).

Several correlations might be noted between receptor distribution or function

and the natural history of the particular animals concerned. Of the two cave crickets providing favorable receptor preparations, *Hadenoecus*, with the more extensively distributed chemoreceptors on the legs, is reported to be more strictly limited to caves than *Ceuthophilus* (Giovannoli, 1933). The selective advantage of highly developed chemical senses in a totally dark environment is obvious. The sensitivity of the chemoreceptors of *Cambarus* to amino acids is undoubtedly related to a diet of decaying meat, and the absence of any response of its receptors to sugars can be correlated with the lack of any behavioral response to sugars by this species. The results with butterflies likewise indicate the existence of a peripheral discrimination mechanism for the chemicals constituting the normal food in this case, sugars.

All of the spike potentials recorded from chemoreceptors were smaller in amplitude than the spikes from mechanoreceptors of the same animal, unless the same receptor cell responded to both types of stimuli. This is in accord with the usual assumption that chemoreceptor fibers are smaller than mechanoreceptor fibers (Dethier, 1953; Hodgson, 1955). The fact that many receptors in *Cambarus*, *Hadenoecus*, and *Amoebaleria* showed spontaneous activity supports another idea believed to be of some general applicability—that spontaneous activity is widespread among sensory cells, and that *any changes* in the frequency or pattern of the spontaneous activity (rather than the mere presence of impulses) may constitute the afferent "message" from the sense organs (Roeder, 1955). The antennal receptors of *Amoebaleria*, showing decreased numbers of impulses during administration of vapors, may illustrate a less common direction of change in spontaneous activity which serves as the afferent message.

The present experiments resolve a discrepancy between the earlier work on the labellar chemoreceptors of the blowfly (Hodgson, Lettvin and Roeder, 1955) and the results obtained by Morita *et al.* (1957, and personal communication) using the butterfly, *Vanessa*. The polarity of the spike potentials recorded from *Phormia* was previously reported as negative, using the present recording method, but positive under similar conditions in *Vanessa*. All the spike potentials recorded from chemoreceptors in the present study resulted from an increase in positivity at the distal tip of the sensory hairs (position of the recording electrode) relative to the base of the same hairs (position of the indifferent electrode), and the contrary polarity reported in *Phormia* was subsequently traced to an error in instrumentation. A precise explanation for the positive spike potentials obtained by this method cannot be given at the present time, but might possibly be explained by generation of the main negative spike potential at the cell body region of the receptor, which would leave the actual chemosensory area with a relatively positive charge. Experiments to localize the main impulse generating area within the receptor are now underway.

The failure to record potentials from chemoreceptors in a large majority of the arthropods tested could result from a real absence of these receptors in the appendages tested or from limitations of the technique. The latter is the more probable explanation in most cases. Particularly unfortunate is the apparent inapplicability of the technique to recordings from the antennae of most insects. Unavoidable short circuits between indifferent and recording electrodes explain some negative results, as noted above, but inability to position the recording electrode over one or a few receptor sensilla and the small size of the spike potentials from the chemo-

receptors undoubtedly account for most of the failures. The optimum preparation for use with this technique appears to be an elongated sensillum, well isolated from surrounding sensilla, and containing very few receptor cells—an ideal approached more conveniently in the labellar chemoreceptors of flies than with any other arthropod preparations yet tested. A similar survey of the chemoreceptors of marine arthropods is planned.

It is a pleasure to acknowledge the courtesy of Dr. Horton H. Hobbs, Jr., Director of the Mountain Lake Biological Station, who facilitated the field work in many ways. Mr. David Bardack assisted in collecting the animals. Drs. V. G. Dethier and K. D. Roeder have been most helpful in critically reading the manuscript.

Summary

1. Electrophysiological tests with externally applied, fluid-filled electrodes were performed upon thirty-seven species representing four classes of arthropods. Afferent chemoreceptor impulses were recorded in animals of five types: a crayfish (*Cambarus*), a millipede (*Pseudopolydesmus*), two orthopterans (*Ceuthophilus* and *Hadenoecus*), a helomyzid fly (*Amoebaleria*), and six species of butterflies.

2. Receptors sensitive to chemical, tactile, and temperature stimuli within normal physiological ranges are found in certain Lepidoptera (*Epargyreus* and *Limenitis*) and Diptera (*Amoebaleria*). Receptors with a dual sensitivity to at least two of the above types of stimulation are found in *Pseudopolydesmus, Ceuthophilus,* and *Hadenoecus.* It is concluded that multiple sensitivities of receptors are not exceptional in arthropods.

3. Chemoreceptors sensitive to amino acids, but insensitive to tactile and temperature stimuli, are found on the chelae and protopodites of the first two walking legs of *Cambarus bartonii sciotensis.*

4. With the present recording method, spike potentials from chemoreceptors represent increases in positivity at the distal tip of the receptor cell, relative to the cell body.

5. Relationships between functional characteristics of chemoreceptors and the natural history of the animals are discussed.

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MORPHOLOGY OF MAIN AND ACCESSORY ELECTRIC ORGANS OF NARCINE BRASILIENSIS (OLFERS) AND SOME CORRELATIONS WITH THEIR ELECTRO-PHYSIOLOGICAL PROPERTIES

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Like other Torpedinidae (Bigelow and Schroeder, 1953), *Narcine brasiliensis* (Olfers) possesses electric organs. While they have previously been studied physiologically (Chagas *et al.*, 1953; Cox and Breder, 1943) no anatomical descriptions seem to have been published of the organs and their innervation, presumably because these have been considered to be identical with the findings in *Torpedo*. In the course of a new study (unpublished data from this laboratory) of the electrophysiology of *Narcine* electric organs with intracellular recording, certain discrepancies were observed which indicated differences between the structure of *Torpedo* organs and those of *Narcine*. A hitherto undescribed, smaller, accessory organ was also found, distinct in its electrophysiological properties from that which will now be called the main electric organ. Anatomical data to be reported here show that the accessory organ also differs from the major organ in structure, innervation, and in the size and orientation of the electroplaques. The present paper reports chiefly the gross anatomical and general histological findings, relating these to the functional properties of electric organs. Data on the embryology and on the fine structure of the electroplaques, including detailed studies of electron microscopic preparations, will be reported elsewhere (Mathewson and Lehrer, and Mathewson and Wachtel, unpublished data).

Materials and Methods

Narcine brasiliensis inhabits the inshore waters of the Atlantic Ocean from Brazil to North Carolina (Bigelow and Schroeder, 1953). It is one of the smallest of the Torpedinidae, the 42 adult specimens brought into the Marineland Research Laboratory for the present study ranging from 20 to 45 cm. in length. In contrast, the larger of the *T. occidentalis* dissected by Hunter (*cf.* Keynes, 1956) measured

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about 1.25 m. Although it seems that specimens of this size have now become rare (Bigelow and Schroeder, 1953), a fish almost 0.75 m. long was brought into the Marine Biological Laboratory at Woods Hole in 1956 (Grundfest, 1957a).

Many of the fish available to us were used chiefly for electrophysiological work, and dissection at this time was confined to a minimum. The electrophysiological data provided clues to probable anatomical structures which were then sought for in other specimens, studied in fresh dissection, or in preserved preparations. Histological material was prepared with a number of techniques for different purposes. The details of these methods will be given in later publications.

Results

A. The Main Organs

Gross anatomy. Superficially the paired, kidney-shaped main electric organs of *N. brasiliensis* (Fig. 1) resemble closely those of the other Torpedinidae (Bigelow and Schroeder, 1953; Fritsch, 1890). They comprise about twenty per cent of the total weight of the fish. Both dorsally and ventrally the main electric organs are in close contact with the skin and their surface area is clearly outlined. However, the patterned pigmentation of the dorsal skin surface of *Narcine* partially obscures this outline. On the ventral surface, not only is the total outline of the organ clearly visible, but also the honeycomb-like arrangement of the columns of electroplaques is seen through the skin. The main organ of each side extends laterally from the outer surface of the gill sacs to a clearly demarcated line near the edge of the pectoral fin. Rostrocaudally the organ starts slightly anterior to the eyes and extends back to the lines of the pectoral girdle.

Columns of electroplaques. As in other Torpedinidae the main organ is made up of a number of closely packed vertical columns, each column composed of electroplaques stacked one atop the other like a roll of coins. The packing of the columns leads to the honeycomb pattern mentioned above, but each column takes on an irregular rather than a hexagonal shape. In 10 adult specimens the number of columns ranged from 386 to 452 (average 419, Table I). This figure falls within the range previously given for this species (Bigelow and Schroeder, 1953; Cox and Breder, 1943; Fritsch, 1890). In late embryos and new-born specimens examined, the number of columns in the main organ was appreciably less than in the adults (Table I). This matter, important in the theoretical aspects of electric organ development, will be dealt with in more detail in the Discussion.

The array of electroplaques in a column. As in other Torpedinidae, the electroplaques in the main organ of *N. brasiliensis* are innervated only at their ventral surfaces. This was established both electrophysiologically (unpublished data from this laboratory) and anatomically. Electrophysiological data, employing successive penetration of the cells in a column with a microelectrode, indicated that the electroplaques were exceedingly thin. This was confirmed on histological preparations, the average thickness of the electroplaques being about 7 μ (*cf.* Fig. 3), although the surface they present is large (1.5 to 2 mm. in diameter, *cf.* Fig. 2). As with many other types of electroplaques, however, there is considerable extracellular material (Ballowitz, 1938; Ellis, 1913; Grundfest, 1957a; Luft, 1956; Szabo, 1956). Thus, there were only 486 to 541 cells (average 495) in the thickest (ca. 2 cm.)

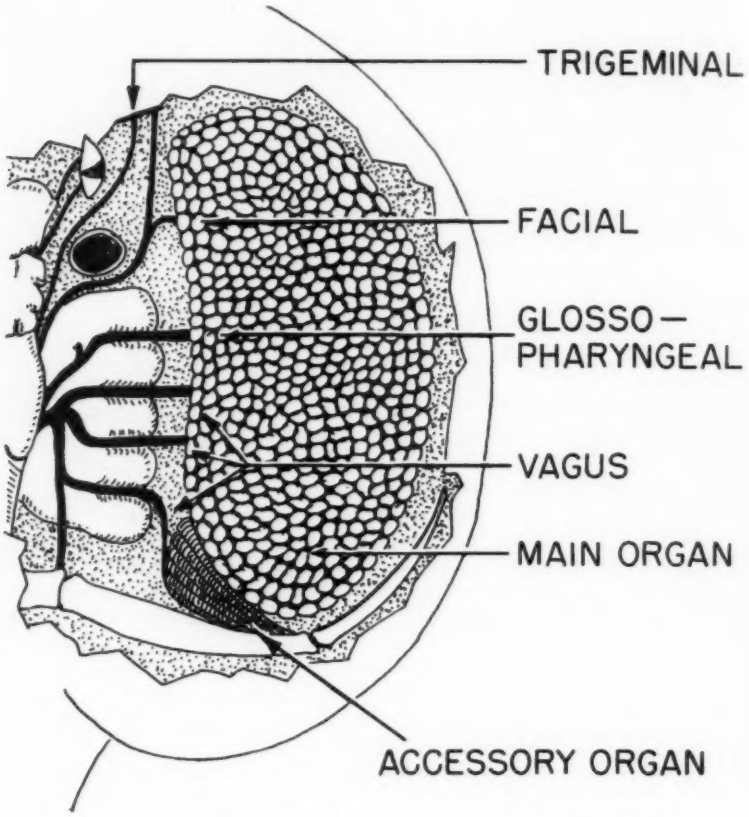

FIGURE 1. Electric organs and their innervation in *N. brasiliensis*. Dorsal view, partly schematic.

TABLE I

Number of electroplaque columns in main organ

Type of specimen	Number of specimens	Number of columns*	Average number
Adult	10	386, 381, 394, 400, 430, 440, 447, 450, 452	419
New-born	3	269, 287, 348	301
Embryo**	5	277, 297, 300, 348, 351	315

* In each fish the columns of one organ were counted three times. The figures given are averages.

** The embryos were all in a very late stage of development.

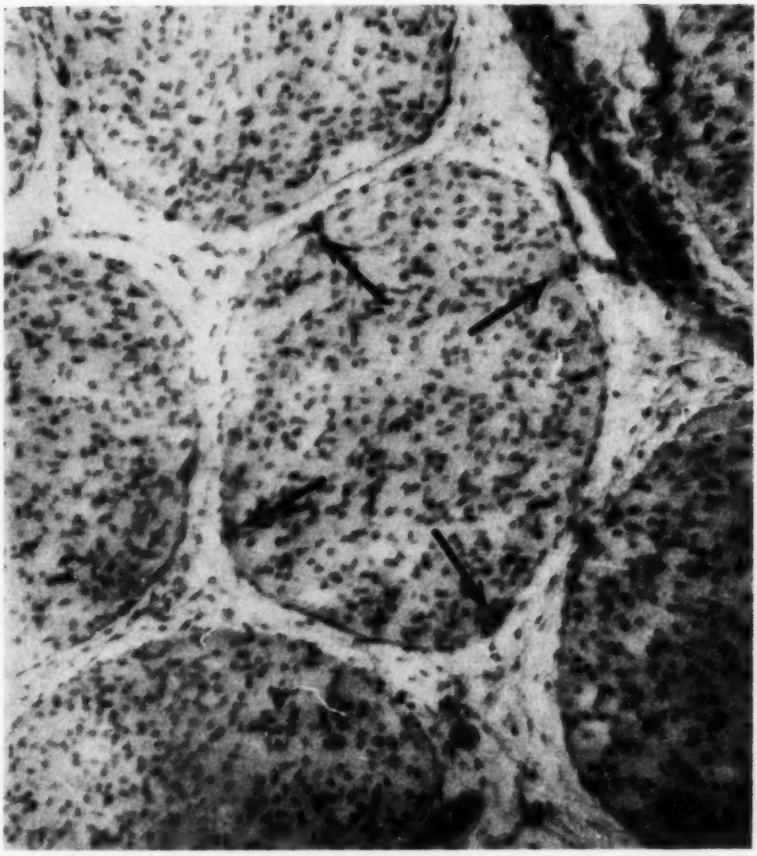

FIGURE 2. Innervation of individual electroplaque. Arrows point to four nerve fibers which become unmyelinated and disperse profusely over innervated surface. Fiber in lower left quadrant probably was cut parallel to its axis. Largest diameter of fiber is 2 μ.

portions of the main organ. In the shorter columns, nearest the outer edge of the fish, the average number of cells was 314.

B. Accessory Organs

Caudad to each main organ in *Narcine* there lies a smaller structure that both by electrophysiological and anatomical criteria has now been identified as another, hitherto undescribed, electric organ. This differs significantly in many respects from the main organ and therefore is termed an accessory electric organ.

Gross anatomy. The accessory organs arise dorsally in the articulation of the cartilaginous scapular process of each side. They run obliquely ventral and slightly

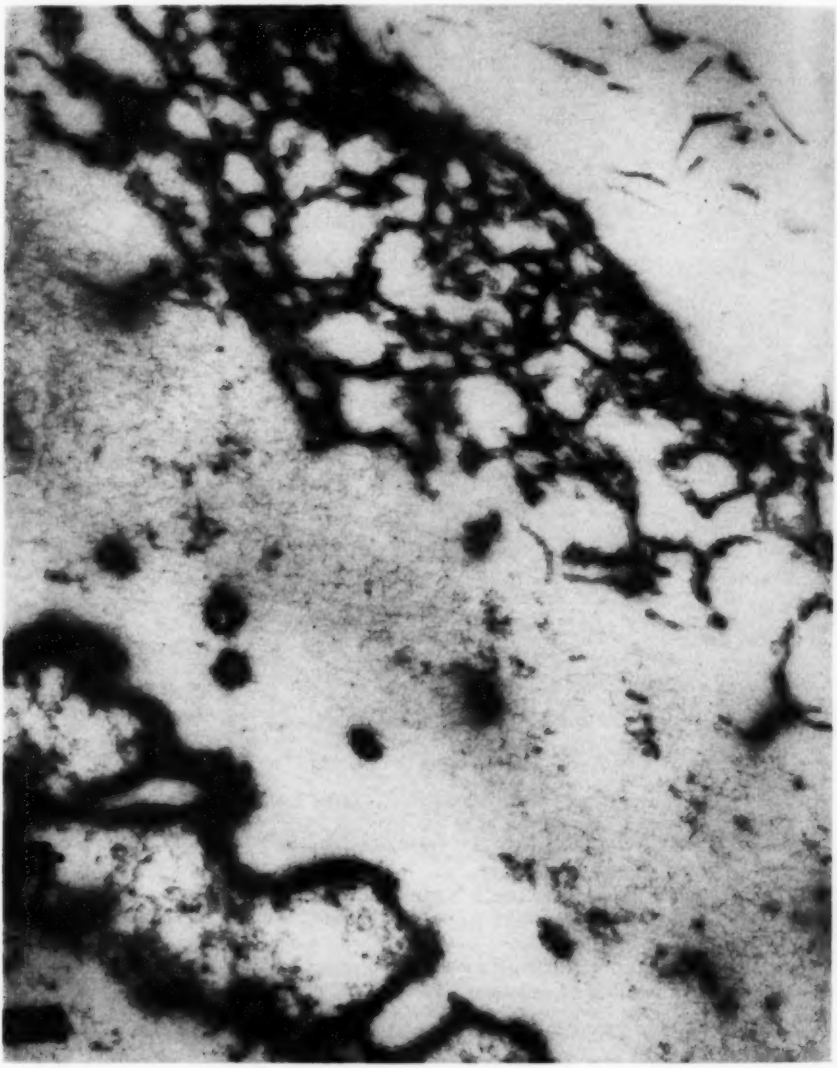

FIGURE 3. Electron micrograph of a slightly oblique cross section of electroplaque. Index (lower left) is 1 μ. Innervated surface, seen diagonally, at lower left, shows nerve fiber following involuted contour of cell membranes. Ring-like structures are sections of infolded fingers of innervated membrane. Interior of electroplaque shows fibrous material. Non-innervated face of cell (upper right) has complex system of interconnected canaliculi. Collagen fibers in extracellular space. Outlines of membranes not clear in this section which was cut thick (0.1 μ) to show other structures.

rostral, toward the midline, terminating in contact with the ventral skin immediately behind the main organs (Fig. 1). Each is about 1 cm. in diameter and about 2 cm. long in 30-cm.-long adult fish. It is separated from the main organ by a distinct layer of connective tissue.

Columns of cells. On the average, only 10 columns are found in the accessory organ. At the ventral surface the columns are tightly compressed and assume a honeycomb appearance which is also visible through the ventral skin. The cells in each are 2 mm. or slightly more in diameter. The area of each cell thus is not much larger than in the main organ and the anterior ventral margin of the accessory organ is not clearly differentiated from the adjacent main organ.

Cellular array in column. A striking feature, further differentiating the accessory organ, is the thickness of the individual cells, which is 20-30 μ, or 3 to 4 times greater than in the main organ. The number of cells in series in a single column is about 200, indicative of the large proportion of extracellular material also in this structure. Another difference between the main and accessory organs is the orientation of the innervation of the electroplaques. In the accessory organ, the dorsal surfaces of the electroplaques are innervated, the discharge of this organ producing positivity at the ventral surface of the fish (unpublished data from this laboratory) whereas discharge of the main organ results in negativity at the ventral surface (*cf.* Grundfest, 1957a). Responses to a single stimulation of the accessory organ produce only 0.5 to 1 volt, in comparison with the 25 to 35 volts generated during the discharge of the main organ.

C. Nerve Supply to the Electric Organs

Gross dissections of *Narcine*, as well as histological preparations, disclose some differences in the neural anatomy between this form and *Torpedo*.[5]

Gross anatomy. In *Narcine* the facial (VII), glossopharyngeal (IX), and vagus (X) nerves supply the electric organ (Fig. 1). The fifth cranial nerve (trigeminal) does not enter the electric organ, although in *Torpedo* it is reported to participate in the innervation (Fritsch, 1890; Szabo, 1955).

The facial, immediately after emerging from the skull, bifurcates into two large trunks. The cranial branch passes around the anterior periphery of the main electric organ and thence radiates throughout the head and pectoral fin. The remaining branch enters the electric organ immediately anterior to the first gill sac. The glossopharyngeal nerve, which emerges from the brain closely associated with the auditory nerve (VIII), leaves the skull ventral to the otic capsule. Passing between the first and second gill sacs, it immediately enters the electric organ. Of the total of four branches which comprise the vagus nerve, the first two pass between the second and third, and the third and fourth gill sacs, respectively, then entering the main electric organ. The third branch passes between the fourth and fifth gill sacs, runs along the anterior edge of the scapular process and enters the accessory electric organ. The fourth branch of the nerve runs parallel to the vertebral column back to a point near the pectoral girdle, then disappears into the musculature of this area.

[5] But one specimen of *Torpedo ocellata* was available for comparison. However, published descriptions of *Torpedo* neuroanatomy are detailed (Bigelow and Schroeder, 1953; Fritsch, 1890; Szabo, 1955).

Innervation within the electric organ. Once having entered the electric organs, the nerves branch profusely in the connective tissue, as has been described for *Torpedo* by Fritsch (1890). Individual electroplaques are, in general, each supplied by four single myelinated fibers (Fig. 2). The fibers lose their myelin sheath close to the innervated surface, and their branching is extremely profuse (Mathewson and Wachtel, unpublished data). The synaptic contacts are dispersed over the innervated electroplaque surface, which is ventral in the main organ and dorsal in the accessory.

Detailed electron microscopic and histochemical studies on the *Narcine* electric organs will be reported elsewhere (Mathewson and Lehrer, unpublished data). Electron micrographs indicate a considerable degree of gross differentiation between the innervated and non-innervated faces of the electroplaques (Fig. 3). The nerves running along the innervated surface often make intimate contact with finger-like inpocketings of this membrane. Vesicular structures (de Robertis and Bennett, 1953; Robertson, 1957) are seen in the cytoplasm of the nerve terminals. At the non-innervated face the cell is profusely riddled by interconnected canaliculi, some of which appear to extend up to the tubules made by the inpocketing of the innervated surface.

Discussion

Distinctions between the main and the accessory electric organs. Several differences in electrophysiological properties have already been indicated. A marked distinction is the reverse polarity of the activity in the accessory organ. The voltage produced by a single stimulation of the accessory organ is small, but grows rapidly upon repetitive stimulation (unpublished data from this laboratory). The large facilitation is in marked contrast to the behavior of the main organ and indicates important differences between the functional synaptic connections of the organs, which may be more clearly revealed after further histochemical and electron microscopic studies.

The anatomical distinctions are also marked, particularly the reversed surface of innervation and the greater thickness of the electroplaques of the accessory organ. The different orientation of this organ, with the columns running obliquely rather than dorsoventrally as in the main organ, and the delineation of the accessory organ by a distinct investment of connective tissue suggest that the muscles from which this organ is derived are different from those which are precursors of the main organ (Fritsch, 1890). Embryological material has not yet been studied sufficiently to reveal that origin.

Number of columns and electroplaques. Our counts of the number of columns in adult fish closely approximate other data in the literature. However, the embryos and new-born fish had a significantly smaller number of columns, although the number of cells in each of these columns was comparable with that in adults. This finding appears to contradict the delle Chiaie-Babuchin rule (du Bois-Reymond, 1881; Grundfest, 1957a) which states that the total number of electroplaques laid down is fixed early in development and does not increase thenceforth. It seems, rather, that the transformation of muscles (or of their anlagen) to electroplaques proceeds at unequal rates, more rapidly in the central portions of the main organ than at its periphery. It has been noted (Grundfest, 1957a) that regeneration of

tissue takes place in knifefishes (Ellis, 1913) and this apparent contradiction of the dell Chiaie-Babuchin rule has been confirmed in this laboratory (unpublished).

In three adult *Narcine* Cox and Breder (1943) obtained an average count of 380 columns (range 343–416). The average in three embryos was 286 (range 264–340). These authors also concluded that the number of columns increased with growth of the fish. In two embryos, they also counted the number of cells in a column, finding an average of 305 in one and 482 in the other. The last, which is close to the value obtained in the present work from adults and embryos (500), supports the conclusion that the number of electroplaques in a column does not increase.

The higher values obtained in the present work help to remove a puzzling difficulty in electrophysiological data (Grundfest, 1957a). On the basis of the older figure for the series elements and of reported maximal discharge voltage, it had been estimated that the EMF of a single electroplaque of *Narcine* was about 120 mv. However, these cells do not produce spikes but only postsynaptic potentials (p.s.p.'s) (Grundfest, 1957a; and unpublished data from this laboratory). Unlike spikes which in skeletal muscle fibers and in eel electroplaques attain amplitudes of about 150 mv, the known varieties of depolarizing p.s.p.'s do not exceed the resting potential, which is generally some 60–80 mv. The maximal discharges in the fish used in the present experiments were about 35 volts in amplitude and this indicates that each electroplaque was capable of a maximal response of about 70 mv. This calculation approximates the resting potentials obtained in the cells, and accords also with direct observations on the responses of single electroplaques (unpublished data from this laboratory). Calculation for the e.m.f.'s of single electroplaques of *Torpedo marmorata* (Grundfest, 1957a) also gave very high values. It is likely that Fritsch (1890) also underestimated the number of cells arrayed in series in each column in these fish. *Torpedo* electroplaques are about 20 μ thick, but are densely packed (Luft, 1956, Fig. 4). It is very unlikely, therefore, that in these larger fish the series array (given as 400) is smaller than that found in *Narcine* (500).

Innervations of the electric organs. It has already been noted that the trigeminal nerve does not supply the electric organs in *Narcine*. According to Fritsch (1890) the *Torpedo* organs are innervated by the trigeminal and vagus nerves. However, he details an extensive controversy regarding this question. Other authors (*cf.* also Rosenberg, 1928) included the facial and hypoglossal in the nerve supply of the organs. Hunter's famous preparation (reproduced in Rosenberg, 1928) shows the electric organ of *T. occidentalis* supplied only by the nerves that would appear to be the glossopharyngeal and the first two branches of the vagus nerve.

Another difference between *Torpedo* and *Narcine* is the finding that the innervation of single electroplaques in *Narcine*, while as regular as is that pictured for *Torpedo* (Fritsch, 1890; Grundfest, 1957a), does not occur at six, but at four points of the cell surface. It seems likely that Fritsch (1890) overemphasized the hexagonal configuration in *Torpedo* and, indeed, in a number of illustrations of his monograph he shows surface views of the columns with shapes that are far from being regular or hexagonal.

Some functional correlations with the anatomical data. The dense synaptic innervation of the individual electroplaques in *Narcine* is in agreement with the similar data reported for *Torpedo* and *Raia* (Ballowitz, 1938; Grundfest, 1957a).

This would appear to have an important functional value. The cells generate only p.s.p.'s which are not electrically excitable and therefore do not propagate (Grundfest, 1957b). A maximal discharge of the organ would be produced only by simultaneous activation of a large proportion of the membrane. This result is achieved by dense synaptic terminations.

The usefulness of this densely innervated surface for a number of experimental purposes (Grundfest, 1957a) is augmented by the finding that the accessory organ has different kinetics of responsiveness to its neural stimuli. As noted above, this will provide a comparison material in the same preparations, not only for electrophysiological studies, but also for correlation of structure and function.

The preliminary electron microscopic data also suggest some possible correlations between function and structure. The current theories of muscle structure and of the contractile process (*cf.* Huxley, 1957) seek to account for transfer of activity from the electrically excitable membrane to the contractile elements by a special membrane extending into the muscle fiber and lying perhaps at the electrically excitable membrane to the contractile elements by a special membrane extending into the muscle fiber and lying perhaps at the Z lines. The dense canaliculi seen in the non-innervated aspect of the *Narcine* electroplaques might be the remnants of these structures, tubules which represent extensions of the membrane. The inpocketings of the opposite, synaptically excitable membrane may be continuous with the segments of the canalicular structures. A rather similar, but not so dense system is also found in eel electroplaques (Grundfest, 1957a; Luft, 1956) and in other types of electric organ (personal communication from Dr. Luft). Examination of electric organs in their various embryological states therefore may furnish important clues to the nature of the transfer from conductile to contractile activity.

Narcine electric organ also may be a favorable object to test current views (del Castillo and Katz, 1956; de Robertis and Bennett, 1953; Robertson, 1957) that the vesicles in the presynaptic terminals may represent concentrations of transmitter agent. Repetitive stimulation of the electric nerve of *Narcine* (Chagas *et al.*, 1953) rapidly blocks the response of the organ. With the period of unresponsiveness is correlated a large fall in the concentration of acetylcholine in the organ. The gradual return of responsiveness is also associated with a rise in the acetylcholine concentration. If the vesicles are the sites of transmitter storage, they should be subjected to marked changes upon repetitive stimulation and during subsequent recovery of responsiveness.[6]

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[6] For the use of the electron microscope and other facilities we are indebted to Drs. Leonard Ornstein and Allen Wachtel of the Cell Research Laboratory, Mt. Sinai Hospital, New York. Thanks are also due to Miss Ruby Tamura for preparing much of the tissue used in this study.

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THE OXIDATIVE METABOLISM OF EGGS OF URECHIS CAUPO[1]

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The cytochrome system is of such widespread occurrence in cells of aerobic organisms that reports of its absence in any particular case are of special interest.

There have been several instances in which spectroscopic detection of absorption bands of the cytochromes was at first reported to be negative and later shown to be positive when improved methods were employed. Thus in eggs of sea urchins Brachet (1934), Lindahl (1936), Krahl, Keltch and Clowes (1939) and Ball and Meyerhof (1940) reported that the cytochrome bands did not show up spectroscopically, although early evidence of inhibition of O_2-uptake by cyanide and by carbon monoxide (Runnström, 1930) indicated the operation of the cytochrome system, at least in the fertilized eggs. Later, Rothschild (1949), Borei (1951) and Yčas (1954), using the method of Keilin and Hartree (1939, 1949) of intensifying cytochrome bands by cooling the material in liquid air, were able to demonstrate the bands of cytochromes *a* and *b*.

It was also thought, at one time, that the respiratory system differed qualitatively in unfertilized and fertilized sea urchin eggs, the cytochrome system being inoperative in the former and brought into play upon fertilization (Korr, 1939). This was based on evidence of insensitivity of the respiration of unfertilized eggs to inhibition by cyanide and carbon monoxide (Runnström, 1930; Lindahl, 1939; Korr, 1937), and reported differences in the effect of temperature on the respiration of unfertilized and fertilized eggs (Rubenstein and Gerard, 1934; Korr, 1937). However, this evidence has now been largely contradicted. Thus, Robbie (1946b) showed that the O_2-uptake of unfertilized sea urchin eggs could be almost completely inhibited by low concentrations of cyanide when the precaution is taken of preventing the distillation of cyanide from the egg suspension to the center-well of the manometer vessel, by the use of appropriate $Ca(CN)_2$–$Ca(OH)_2$ mixtures in the center well. In regard to the effect of carbon monoxide on unfertilized sea urchin eggs, Rothschild (1949) was able to demonstrate a photo-reversible inhibition of O_2-uptake when account was taken of a CO-induced stimulation and a light-induced inhibition of respiration. Concerning the effect of temperature on respiratory rates, further measurements (Tyler and Humason, 1937; Borei and Lybing, 1949) have shown no significant differences between unfertilized and fertilized sea urchin eggs.

In eggs of the echiuroid worm *Urechis caupo*, a failure to detect the absorption bands of cytochrome was reported by Horowitz and Baumberger (1941). In these eggs there is a reversibly autoxidizable pigment which Horowitz (1940a)

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called urechrome. From the facts that both the oxidized and reduced states are observed naturally in the eggs, depending upon the presence or absence of oxygen, that the pigment is reducible by the cells, and that it autoxidizes in the physiological range of pH, he concluded that it was probably involved in the cellular respiration. Upon further characterization Horowitz and Baumberger (1941) suggested that the pigment was related to the hemins. Its chemical constitution has not as yet been determined.

For these and other reasons we decided to examine the O_2-uptake of fertilized eggs of *U. caupo* in the presence of cyanide and of carbon monoxide, and to examine the eggs spectroscopically using the low temperature method of Keilin and Hartree (1939). Previous measurements of the respiration of this material were made for other purposes (Tyler, 1936; Tyler and Humason, 1937; Tyler and Horowitz, 1938; Horowitz, 1940b) and it was noted that the fertilized eggs have rather consistent values for their absolute rate of O_2-uptake, although that of the unfertilized eggs may vary greatly from one batch to another. Some preliminary experiments with cyanide and azide were mentioned in the report of a seminar talk (Tyler, 1937). These were for the purpose of investigating possible correlations between cleavage retardation and respiratory inhibition and were done before the introduction by Robbie (1946a) of the $Ca(CN)_2$–$Ca(OH)_2$ center-well mixtures for preventing loss of cyanide from cell-suspensions in the manometer vessels. The preliminary experiments indicated, however, that inhibition of respiration by cyanide was obtainable in these eggs.

Material and Methods

General manometric procedure. Eggs of *U. caupo* were inseminated and washed in sea water buffered at pH 8 with 0.01 *M* glycylglycine, which Tyler and Horowitz (1937) showed to be a suitable non-injurious agent to replace the bicarbonate system of ordinary sea water. The latter system does not provide satisfactory buffering because the absorption of CO_2 by the alkali in the manometer vessels occasions a rise in pH of the sea water which is only partially and variably compensated by the CO_2 production of the respiring cells.

After examination to check that fertilization had been successful, 3- or 4-ml. aliquots of egg-suspension were transferred to standard Warburg-Barcroft manometer flasks whose calibration volumes were around 20 ml. Readings were taken after a 30-minute equilibration period in the water-bath, the shaker speed being 95 c.p.m. at 4 cm. stroke. The temperature was 20° C.

Cyanide experiments. Robbie's (1946a) $Ca(CN)_2$–$Ca(OH)_2$ mixtures, in 0.6-ml. quantity, were used in the center-wells of the manometer vessels in order to establish and maintain known concentrations of cyanide in the egg suspensions, and provide sufficient alkali to absorb the respiratory CO_2. Fluted filter papers were used in the center-wells to increase the absorbing surface. A stock 1.32 *M* calcium cyanide solution was prepared according to Robbie and Leinfelder (1945) and this was diluted with 10% $Ca(OH)_2$ according to Robbie's (1946a) figures to provide center-well mixtures establishing the following concentrations of HCN in the experimental fluid at 20° C.

HCN molarity	10^{-3}	10^{-4}	5×10^{-5}	10^{-5}	5×10^{-6}
Molarity of $Ca(CN)_2$ in 10% $Ca(OH)_2$	0.38	0.046	0.023	0.0054	0.0028

In some experiments an appropriate quantity of NaCN was added to the egg-suspensions in the manometer flasks just before the beginning of the experiment. In the latter case the equilibration-time was reduced from thirty to fifteen minutes. Manometer flasks and other vessels containing cyanide solutions were kept stoppered at all times except when eggs were added and the flasks were put on the manometers.

CO experiments. The gas phase of the manometers was filled with 95% CO in O_2 (95% CO/O_2), after flushing out the air. Ninety-five per cent N_2/O_2 and air controls were run at the same time. The center wells contained 0.3 ml. N/1 KOH and filter papers. Equilibration was in the dark for ten minutes.

Results

Cyanide experiments. The results of three sets of experiments were clear-cut in the sense that, even at low concentrations, cyanide inhibited the respiration of fertilized eggs. Data from one of these are plotted in Figure 1. The lines labelled

Table I

The effect of cyanide, added 20 to 25 minutes after fertilization, on the percentage development of eggs of Urechis caupo, examined at 3 hours. The sea water contained 0.01 M glycyl glycine, pH 8.0, T° C. 20

Conc. HCN	Uncleaved	2-cell	4-cell	8-cell	16–32 cell	Unfertilized
10^{-4} *M*	99 } polar bodies					1
$5 \cdot 10^{-5}$ *M*	99 }					1
10^{-5} *M*	49	30	20			1
$5 \cdot 10^{-6}$ *M*	2	1	4	46	46	1
0	3				96	1

O,KOH and $O,Ca(OH)_2$ were controls to compare the CO_2-absorptive powers of 10% KOH and $Ca(OH)_2$ in the center-wells of the manometer flasks. As this and other tests showed, the $Ca(OH)_2$ proved as effective as the KOH in absorbing CO_2 under the conditions of these experiments.

Table I shows the effects of the different concentrations of cyanide on the development of the eggs when examined at the end of the experiment.

Carbon monoxide experiments. The results of an experiment in which just-fertilized eggs were subjected to 95% CO/O_2 and 95% N_2/O_2 are shown in Figure 2, in which periods of illumination and darkness are indicated by black and white blocks along the time axis. If the rate of O_2-consumption [3] in the curve labelled CO/O_2 is examined by itself, it is clear that it rises upon illumination and falls in darkness in the manner considered characteristic of cytochrome-catalyzed respiration. When, however, comparison is made between the curve labelled CO/O_2 and the control labelled N_2/O_2, it is equally clear that, in the light, CO also stimulates the gas-uptake of these eggs. Illumination had no inhibitory effect on the O_2-uptake of eggs in equilibrium with air.

Table II shows the effect of CO in this experiment on egg development. The

[3] The use of the terms O_2-consumption, O_2-uptake, and respiration in the description and discussion of the CO-experiments is subject to the qualification that there is the possibility (see Discussion) that some of the gas consumed might be CO.

inhibition is not so marked as in the cyanide experiments, but it might, of course, be more dramatic if higher CO tensions were used.

The results of six sets of experiments with 95% CO/O_2 and 95% N_2/O_2 are presented in Table III. The last two columns of the table give a measure of the effect of CO on the respiratory rate, in the dark and in the light, based on lateral

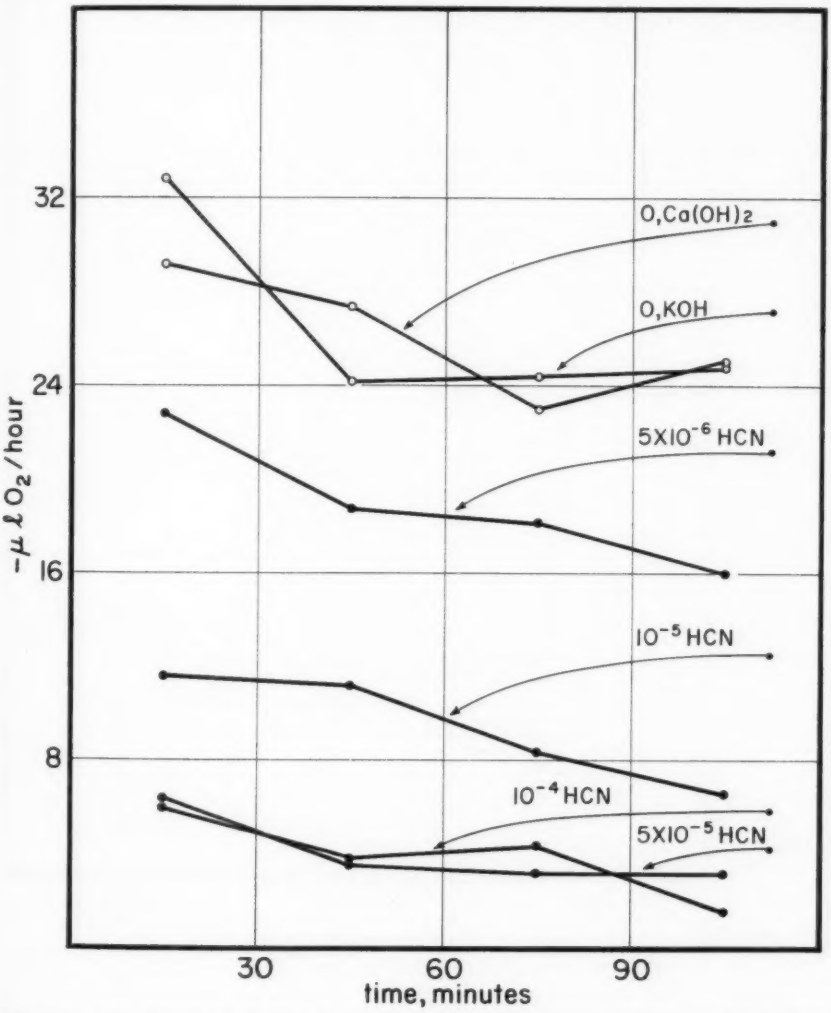

FIGURE 1. The respiration of eggs of *Urechis caupo* in the presence of HCN. For further details see text.

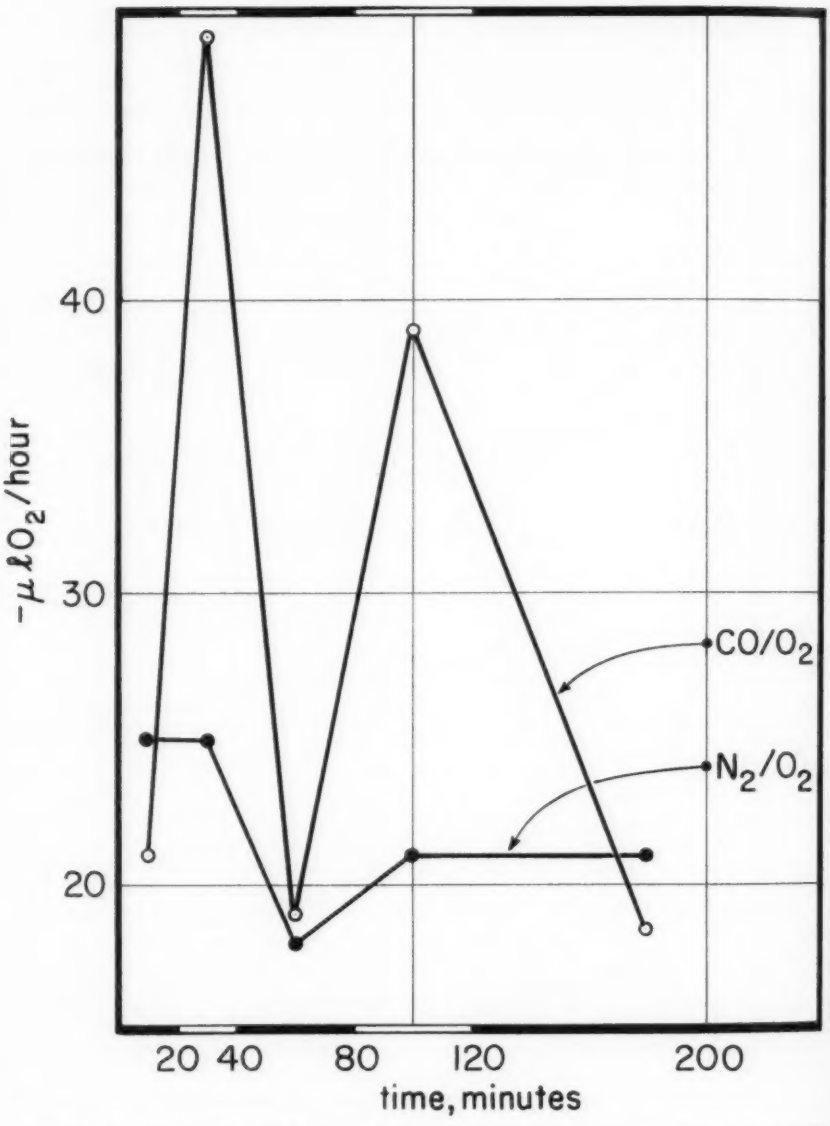

FIGURE 2. The oxygen uptake of eggs of *Urechis caupo* in the presence of 95% CO in O_2 and of 95% N_2 in O_2. The black and white blocks along the time axis correspond to periods of darkness and illumination. For further details see text.

TABLE II

The effect of 95% CO in O_2 and 95% N_2 in O_2 on the percentage development of eggs of Urechis caupo, exposed at ¼ hour and examined at 5 hours after fertilization. The sea water contained 0.01 M glycyl glycine, pH 8.0, T° C. 20

Gas	Uncleaved	64-cell	128-cell
Air	15	50	35
N_2	15	50	35
CO	15	85	

TABLE III

Effect of carbon monoxide on the respiration of eggs of Urechis caupo in the light and in the dark (All experiments started about 40 minutes after fertilization. Temp. 20° C.)

Experiment	Respiration period		Cu.mm. O_2 per hr. per 10^3 eggs		Ratios $\frac{\text{Resp. in 95\% CO–5\% } O_2}{\text{resp. in 95\% } N_2\text{–5\% } O_2}$	
			95% CO–5% O_2	95% N_2–5% O_2	Dark	Light
1	0′–15′	dark	7.6	9.7	0.78	
	15′–30′	light	14.6	9.7		1.59
	30′–45′	light	13.5	8.2		1.65
	45′–60′	dark	5.9	7.7	0.77	
	60′–75′	dark	4.9	6.7	0.73	
2	0′–20′	light	15.7	9.8		1.60
	20′–60′	dark	7.1	9.1	0.78	
	60′–100′	light	19.5	9.3		1.88
	100′–140′	dark	7.8	8.8	0.89	
	140′–160′	light	15.7	9.8		1.60
3	0′–21′	dark	7.5, 7.6	9.4, 8.7	0.83	
	20′–40′	light	17.1, 18.3	8.0, 10.1		1.95
	40′–80′	dark	6.8, 6.8	7.2, 6.1	1.02	
	80′–120′	light	13.5, 15.1	6.9, 8.4		1.87
	120′–140′	dark	6.9, 6.6	8.0, 7.5	0.87	
4	0′–30′	dark	8.7	11.8, 10.8	0.77	
	30′–60′	dark	9.6	11.1, 14.7	0.75	
	60′–90′	light	18.4	8.2, 9.3		2.10
	90′–120′	light	17.6	8.8, 8.5		2.02
	120′–240′	light	15.2	8.9, 8.9		1.71
	240′–270′	light	14.4	8.9, 8.5		1.66
	270′–300′	light	15.2	10.4, 10.0		1.49
5	0′–90′	light	15.8	6.4		2.47
	90′–150′	light	13.0	5.7		2.28
	150′–180′	light	14.1	5.7		2.47
	180′–240′	light	13.8	6.5		2.12
6	0′–60′	light	13.5	9.4		1.44
	60′–210′	light	15.4	10.9		1.41
	210′–240′	light	13.5	7.4		1.82
	240′–270′	light	11.8	5.7		2.07
	270′–300′	light	15.2	9.9		1.53

comparisons (*i.e.*, of different vessels run in parallel with aliquots of the same egg-suspension). In the dark the respiratory rate in 95% CO/O_2 is consistently lower than in 95% N_2/O_2. Rigid statistical treatment would be complicated because of the differences in times of readings, magnitude of respiration, etc., in the different experiments. However, a simple averaging of the percentage decrease (with double and quadruple weights for experiments 4 and 3, respectively) gives a 15 per cent inhibition of respiratory rate in 95% CO/O_2 in the dark.

Similarly calculated, there is in these experiments, in the light, an 85 per cent average increase in respiratory rate of the eggs in 95% CO/O_2 over that of the parallel controls in 95% N_2/O_2. The figures in Table III also show, for individual manometer vessels, the great effect of alternate light and dark periods on the respiration of the eggs in 95% CO/O_2 and the lack of significant effect of light and dark periods on the respiration of the eggs in 95% N_2/O_2.

Spectroscopic examination of eggs. We have examined the unfertilized eggs of *Urechis* with a narrow-dispersion hand spectroscope (Keilin, 1925) at the temperature of liquid nitrogen, the eggs being suspended in 50% glycerol (*v/v*) with sodium dithionite added (Keilin and Hartree, 1939, 1949, 1955). A double absorption band at 551 mμ, which is in the region of the α-band of cytochrome *c*, could be clearly seen. A further, faint, absorption band at 580–590 mμ (cytochrome *a*) was also seen. The presence of these absorption bands was confirmed by Professor D. Keilin and Dr. R. Hill.

Reduced cytochrome *c* was rapidly oxidized by egg brei in phosphate buffer. A peculiar phenomenon was observed during examination of the oxidation of cytochrome *c* by egg brei. When the oxidized cytochrome *c* and egg brei was kept in comparative darkness and then illuminated through the microscope sub-stage condenser (which automatically occurs during spectroscopic examination), the absorption bands of reduced cytochrome *c* gradually reappeared. This also was confirmed by Professor D. Keilin and Dr. R. Hill. The most probable interpretation is that in the presence of light, some reducing substance is produced by the eggs, causing the reduction of cytochrome *c*. This phenomenon may have some connection with the inhibitory action of light on the respiration of sea urchin eggs (Rothschild, 1949), though, as mentioned above, we have not observed any comparable light-inhibition of respiration in *Urechis* eggs. Certain dyes are affected by light in ways which would be consistent with the observed reduction of cytochrome *c* in light, which raises the possibility that urechrome may be concerned in the phenomenon. For example, Equ. (3) in Clare's article in Hollaender's *Radiation Biology*, Vol. III (1956)

$$DH_2 + O_2 \rightarrow H_2O_2 + D,$$

if written in the form

$$DH_2 + 2\ \text{cyt.c}^{3+} \rightarrow 2\ \text{cyt.c}^{2+} + D + 2H^+$$

is suggestive in this connection.

Discussion

In the introduction to this paper, reference was made to Horowitz's (1940a) view that urechrome and not cytochrome catalyzed the respiration of *Urechis* eggs;

this opinion was based on the facts that urechrome is reversibly autoxidizable and that no absorption bands of cytochrome were observed. We have now shown that the absorption bands of cytochrome are present in these eggs and that an egg brei can oxidize reduced cytochrome *c*. Moreover, the inhibition studies with cyanide and carbon monoxide support the view that the respiration of these eggs is cytochrome-catalyzed. Just where urechrome fits into the picture is, at present, uncertain. The effects of CO and cyanide on this pigment have not, as yet, been studied.

The stimulating effect of carbon monoxide on respiration has been noted in many experiments with eggs and other tissues. The following citations from the literature on this subject will serve to illustrate the widespread occurrence of the phenomenon.

Runnström (1930) found that the respiration of unfertilized eggs of *Paracentrotus* and *Arbacia* was either not inhibited or somewhat higher in carbon monoxide-oxygen mixtures than in air, while that of the fertilized eggs was greatly inhibited. Presumably, although not explicitly stated, these experiments were run in the dark.

Lindahl (1939) obtained a 44% stimulation of the respiration of unfertilized eggs of *Paracentrotus* by 75% CO/O_2 in the dark, and this increased (to ca. 100%) upon illumination. With decrease in oxygen tension to 5% (+ 15% N_2 and 75% CO) the stimulation decreased. For freshly fertilized eggs in the dark he obtained a slight stimulation in 75% CO/O_2 and a marked inhibition in 95% CO/O_2. In the light the fertilized eggs showed marked stimulation by 75% CO/O_2 and this effect decreased as the O_2 concentration was dropped to 5% at constant CO.

Rothschild (1949) measured the respiration of unfertilized eggs of *Psammechinus miliaris* in various $CO–O_2$ mixtures. In 14 comparisons of the effect of 95% CO/O_2 with 95% N_2/O_2 in the dark there was no difference in two, an 11% decrease in three and a 14% increase in nine. Twenty-four comparisons of the effect of 95% CO/O_2 in dark with that in light showed a 44% increase in the light. At the same time he found an inhibitory effect of light on the respiration of the unfertilized eggs in air. This averaged 38% in 44 experiments. With 80% CO/O_2 in the dark there was an average of 55% increase in respiration above that in 80% N_2/O_2, and no significant change upon illumination.

In the ascidian *Phallusia mammillata* Minganti (1957) found an increase in respiration of the unfertilized eggs in 95% CO/O_2 in the dark and a further increase in the light. The fertilized eggs showed a 14% to 20% decrease in the dark, which is about the same degree of inhibition as in the present experiments, and an increase (up to 40%) in the light.

Bodine and Boell (1934) obtained CO-stimulation of respiration of diapause embryos of the grasshopper *Melanoplus differentialis* and no significant effect of light. A similar stimulation by CO was found by Wolsky (1941) in a bivoltine race of the silkworm *Bombyx mori*, but not (Wolsky, 1938) in pupae of *Drosophila melanogaster*. Wolsky (1938) attributes this difference to the pupal stage being one of great activity as compared with diapause. Schneiderman and Williams (1954) found that the respiration of diapausing pupae of the *Cecropia* silkworm was but slightly affected by high concentrations of carbon monoxide; further experiments (Harvey and Williams, 1958) demonstrated that a cytochrome system functioned in this material, the resistance to CO being accounted for by cytochrome oxidase being present in great excess relative to cytochrome *c*.

In non-embryonic tissue the most extensively studied examples of CO-stimulation of respiration were those first reported by Fenn and Cobb (1932a, 1932b) in skeletal and heart muscle of frog and rat. This stimulation occurs in the dark or diffuse daylight and, as shown by Schmitt and Scott (1934), is increased by strong illumination. Fenn and Cobb (1932b) adduced evidence to show that the CO was oxidized to CO_2 and this has been further substantiated by Clark, Stannard and Fenn (1950) by the use of isotopically labelled CO. The latter investigators (1949) also reported such oxidation of CO by the intact animal (turtles and mice).

In plants Daly (1954) obtained increases of about 20% to 30%, in 95% to 97% CO, with leaf tissue of the wild plum, *Prunus americana,* in the dark. From the results of experiments with labelled CO he concluded that the increased gas-uptake by the tissue represents a real stimulation of respiration rather than oxidation of CO to CO_2. He also found a rather high R.Q. (up to 1.33) for the extra gas consumed and therefore suggested that aerobic glycolysis was increased by CO to a greater degree than O_2-uptake. He cited cases of such stimulation of aerobic glycolysis by CO which have been reported in spinach (Ducet and Rosenberg, 1952 [4]), carrot (Marsh and Goddard, 1939), and rat retina and mouse [4] chorion (Laser, 1937).

The above-mentioned investigations indicate that the stimulating action of CO on respiration is of wide incidence in cells and tissues of animals and plants. In some cases (skeletal and heart muscle of frog and rat) there is strong evidence that the extra gas-uptake is due to the oxidation of CO. In others (plum leaves) it appears to be due to the stimulation of endogenous respiration. In the case of the fertilized *Urechis* eggs, and the other cases that have been cited above, the mechanism of the stimulating action of CO is, as yet, unknown and would constitute an interesting area of further investigation. For the present purpose the demonstration of a light-sensitive action of CO on the gas-uptake of the *Urechis* eggs serves to support the other evidence presented that a cytochrome system is operative in this material.

One of us (R.) is indebted to the Biology Division, the California Institute of Technology, for their hospitality during the course of these experiments. We are indebted to Miss Mary Jones for technical assistance.

Summary

1. The respiration and normal development of fertilized eggs of *Urechis caupo* are inhibited by low concentrtaions of HCN, 5×10^{-6} *M*. Known concentrations of HCN were established within the manometer flasks by the use of $Ca(CN)_2$–$Ca(OH)_2$ mixtures in the center-wells, with and without the appropriate amounts of NaCN in the egg suspensions.

2. The respiration of fertilized eggs was photo-reversibly inhibited by 95% CO in O_2. The inhibition of development was not so marked at this tension as in the cyanide experiments.

3. CO markedly stimulated the respiration of the eggs in the light. The occurrence of a similar action in the dark is presumed to account for the moderate degree of depression of respiration by CO in the dark.

[4] Daly (1954) cited a 1951 paper instead of the 1952 paper listed here; also he referred to chicken chorion whereas Laser (1937) refers to mouse chorion.

4. Spectroscopic examination of the eggs at the temperature of liquid nitrogen revealed absorption bands at 551 mμ and 580–590 mμ. Absorption bands at these wave-lengths are associated with the presence of cytochromes *c* and *a*.

5. An egg brei rapidly oxidized reduced cytochrome *c*, but intense illumination of the system reversed the process.

6. It is concluded that the respiration of *Urechis* eggs is cytochrome-catalyzed.

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REGENERATION OF BUDS IN BOTRYLLUS[1]

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The process of budding in the colonial ascidian, *Botryllus schlosseri*, has been carefully analyzed by N. J. Berrill (1941a) and recently by Sabbadin (1955). The new buds (Z_3) arise from the atrial epithelium and epidermis of large buds (Z_2) in which internal structure is nearly complete but which are still attached to the parent (Z_1). The disc-like thickening of the atrial epithelium increases in cell number and area until a certain size, called the maximum disc, is reached. It then folds out into a hemisphere and finally to a closed sphere attached to the large bud by a stalk. Three generations are thus present and connected together at one time. The sphere then goes through a process of expansion, folding, and evagination to form the internal structure of the new zooid. The bud continues to grow until it reaches a size nearly equal to the parent, at which time the latter degenerates and the bud becomes functional. There is considerable variation among colonies in the number of buds formed and the number which reach maturity.

Berrill (1941a, 1941b, 1945) has shown that in young colonies the diameters of the maximum disc and sphere stages are less than half those of older colonies and that they gradually increase with each successive generation. The size of the adult zooid is closely related to the size of the bud and hence to the number of cells initially present. A sphere with a diameter of 0.035 mm., for example, becomes a zooid with a length of 1.1 mm., while a sphere of 0.080 mm. becomes a zooid of 2.6 mm. Sabbadin (1956a, 1956b) showed that the growth of the bud is conditioned not only by its initial dimensions, but also by the quantity of food made available to it by the regression of the parent zooid and the duration of its growth period. In his experiments all but one bud was removed from each zooid in the experimental colonies. These buds and the zooids from them attained greater maximum length than corresponding buds and zooids in control colonies. Sabbadin concludes that the buds on one zooid compete for food made available by the parent as it regresses. This does not explain, however, the gradual increase in size of the zooids with each generation. It was deemed of interest to determine whether the size of the zooid depends directly on the number of cells present in the early bud or is determined in some other manner by the parent. In order to study this problem, buds were damaged at an early stage and the amount of regeneration as shown by the final size was noted.

This degeneration of the parent zooid has generally been thought to be due to the increasing need of the bud for space and nourishment (Berrill, 1935). To test

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whether this is true the buds were removed in an attempt to prolong the life of the zooid.

Materials and Methods

Adult colonies of Botryllus were collected from the dock in Eel Pond at Woods Hole, Massachusetts, and placed in finger bowls on a table of running sea water. Each day a few of the tadpole larvae were released from the colonies and these were collected and placed in Syracuse dishes for approximately 12 hours. After the tadpoles had attached to the glass, the Syracuse dishes were inverted in wooden racks placed in tanks of running sea water. The tadpoles metamorphosed in less than one day, forming oozoids with large right buds which became the first blastozooids. Under these conditions at 22.5 ± 0.5° C. the adult zooid persisted for from 4 to 6 days, with as much as 24 hours' variation between two colonies in the same dish. The experimental colonies were examined every day or two with a binocular dissecting microscope and rough sketches were made to follow the fate of individual zooids and buds.

Buds were removed by cutting through the stalk with a needle sharpened to a blade. With care this could be done with very little damage to the parent, but sometimes the latter was damaged severely and disappeared. To determine whether the new buds originated from the same area as the destroyed buds or from bud primordia posterior to it, all the small buds (Z_3) were cut off 220 large buds (Z_2) in 31 colonies. The site of formation of the new buds was then observed. In 5 colonies an attempt was made to keep the parent zooids from degenerating by constantly removing new buds as they appeared.

Buds were damaged with sharpened steel needles inserted through the tunic. An effort was made to destroy half or more of the forming bud. Although the amount of actual damage varied from bud to bud, in most cases at least half of the bud was destroyed. Frequently, part of the bud was torn away and could be seen sticking to the needle. In preliminary experiments on 21 colonies, buds ranging from the sphere stage to those with some internal structure present were damaged. These were watched to see whether they reached maturity, but no measurements were made to determine if they were full size. In order to examine the effect of destroying approximately half of the cells at a stage before the closed sphere, both right and left buds were damaged when the atrial epithelium had begun to fold out into a hemisphere (between stages 2 and 3 of Berrill, stage 2 Sabbadin). The length of the zooid which formed these buds was then measured with an ocular micrometer and compared with the length of undamaged zooids. The width of the zooids varied in different colonies of the same age and seemed to decrease as the number of zooids around the cloaca increased; therefore, no measurements of width were made.

Results

a) *Degeneration of parent zooids*

In no case observed did an adult zooid persist beyond 24 hours of the time of degeneration of other zooids of the same age. When all the buds were removed from a zooid, that zooid degenerated at the same time as the rest of the zooids in that colony or in other colonies, whether new buds formed or not. The five col-

onies in which new buds were constantly removed as they appeared degenerated and disappeared within 24 hours of the time of degeneration of control colonies.

b) *Formation of new buds*

If the large bud which normally occurred on the right side of an oozoid was removed, a bud then appeared in four cases out of five on the left side of the oozoid and became a normal blastozooid. In later generations, if all the large buds (Z_2) with complete internal structure were cut off (4 colonies), the colony degenerated. If, however, only approximately half of the larger buds were cut off (5 colonies), those remaining proceeded to maturity, and in addition a few new buds appeared. When all the small buds (Z_3) at the sphere stage were cut off 220 large buds (Z_2) in 31 colonies, a total of 72 new buds appeared, an average of one bud for every three parents. There was a great deal of variation among the colonies, with anywhere from zero to seven buds produced by the six or seven parents. Of the new buds, 26 appeared on the left side of the blastozooids and 29 on the right side. Of the latter 6 definitely were from the same area as the destroyed bud, 18 were probably from this area, and 5 appeared posterior to the destroyed bud. The origin of the other 17 buds was impossible to determine. Most of these were first seen in the midst of a degenerating colony quite separate from any blastozooid.

c) *Bud regeneration after damage*

In preliminary experiments in which 107 buds ranging from the sphere stage to those with some internal structure present were damaged, 55 reached maturity. The rest of the buds became progressively smaller and eventually disappeared. In 36 colonies in which 361 hemispheres were damaged, 40% reached maturity as compared to 78% in 8 control colonies with 124 hemisphere stages.

TABLE I

Size regulation of zooids in partially damaged colonies

Colony number	No. of zooids in colony	No. of zooids damaged	Average length of *all* zooids in colony, in mm. ±S.D.	Average length of *damaged* zooids in colony, in mm. ±S.D.	Average length of *undamaged* zooids in mm. ±S.D.
A12c	12	5	1.5 ± .1	1.6 ± .1	1.5 ± .1
A12e	7	3	1.8 ± .1	1.8 ± .2	1.9 ± .1
A12h	21	8	1.9 ± .1	2.0 ± .1	1.8 ± .1
A12k	13	6	1.6 ± .1	1.6 ± .1	1.6 ± .1
A12l	17	4	1.4 ± .1	1.5 ± .1	1.4 ± .1
B2d	25	4	1.8 ± .2	1.8 ± .1	1.8 ± .2
A5a	8	3	1.8 ± .1	1.8 ± .2	1.9 ± .1
A5e	15	13	1.6 ± .1	1.6 ± .1	1.6 ± .1
A5i	7	6	1.8 ± .1	1.8 ± .1	1.9
A1a	16	16		1.8 ± .2	
A5b	6	0			1.4 ± .1
A5g	13	0			1.6 ± .1
A5h	13	0			1.7 ± .1
A6a	7	0			1.8 ± .1
A6b	11	0			1.7 ± .1

TABLE II

Size regulation of zooids in experimental and control colonies

Colony number	No. of Z_1	Length of Z_1 in mm. ±S.D. at time of experiment	No. of Z_2	Length of Z_2 in mm. ±S.D. 1–2 days after reaching maturity	No. of Z_3	Length of Z_3 in mm. ±S.D. 1–2 days after reaching maturity	Length of Z_4 in mm. ±S.D. 3–4 days after reaching maturity
Experimental Colonies							
A3e	9	1.7 ± .1	8	2.0 ± .2	12	2.0 ± .2	2.4 ± .3
A7a	5	1.8 ± .1	8	2.0 ± .1	12	2.2 ± .1	2.8 ± .1
A8a	7	1.7 ± .1	4	1.7 ± .1	4	1.9 ± .1	2.4 ± .1
A10a	4	1.6 ± .1	4	—	8	2.2 ± .1	—
A10e	—	—	6	1.7 ± .1	10	1.8 ± .1	—
Total	25	1.7 ± .1	30	1.9 ± .2	46	2.1 ± .2	2.6 ± .3
Control Colonies							
A3d	6	1.8 ± .1	8	1.8 ± .2	13	1.8 ± .1	—
A7b	5	1.6 ± .2	9	1.8 ± .1	15	1.9 ± .1	2.3 ± .2
A8a	7	1.8 ± .1	10	2.0 ± .1	17	2.0 ± .1	2.6 ± .1
A10b	5	1.8 ± .1	5	1.8 ± .1	11	2.1 ± .1	—
A10f	—	—	5	1.7 ± .1	9	1.8 ± .2	—
Total	23	1.7 ± .1	37	1.8 ± .2	65	1.9 ± .2	2.4 ± .2

d) *Size regulation in damaged buds*

In the first experiments, only some of the hemispherical buds in each colony were damaged with the idea of using the others as controls. The data for 15 such colonies are given in Table I. In colonies A12h and A121 the damaged left buds did not survive, so the right buds measured received the full food supply from the parents. In all other colonies as many damaged left buds survived as undamaged, so the supply of food did not affect the results. The colonies are not all of the same size or age at the time of the experiments, so the average length for different colonies cannot be directly compared, but the average lengths of damaged and undamaged zooids in the same colony show no significant difference.

In later experiments, all the hemisphere stages in a colony were damaged and these colonies were compared with control colonies. Three generations were present at the time of the experiment: the parent zooids (Z_1), the large buds (Z_2), and the hemispherical buds (Z_3). Each of these was measured as it in turn reached maturity. In both experimental and control colonies, most of the left buds reached maturity, so the food supply was about the same for all buds. The data for these experiments are given in Table II. No significant difference can be seen between the experimental and control colonies.

DISCUSSION

If the degeneration of the adult zooid is due only to the increasing need of the growing buds for space and nourishment, removal of all the buds in a colony ought

to have prolonged the life of the zooids. In this study, any attempt to postpone degeneration of the zooid in this way met with failure. No zooid was observed to persist more than 24 hours longer than other zooids of the same age even if its buds were continually removed. Sabbadin (1956b), however, found that when all but one bud was removed from each zooid, that zooid had a prolonged stage of functional maturity. Perhaps removal of all buds was a shock to the zooid and partially caused its regression; however, it seems likely that adult regression will occur without the presence of buds. At the same time the buds may play an important part in the process by their increasing need for nourishment.

There is some question as to the origin of new buds after removal of these already growing. Blastozooids have two potential budding areas, one on the right side and one on the left, although frequently only the bud on the right side reaches maturity. At times, a third bud may be formed posterior to the bud normally found on the right side (see Watterson, 1945, and Sabbadin, 1956a, for a discussion of the number of buds usually formed). It would appear that a certain amount of atrial epithelium is set aside for bud formation; after that is used no more buds can be formed. Frequently in these experiments, when buds were removed from the blastozooid, new buds were formed at the same area as the buds were destroyed. Sabbadin (1956a) reports that he never observed buds arising "de novo" after removal of buds present. Sometimes, however, after he had removed buds in the hemisphere stage, he saw fragments adhering to the atrial side of the parent zooid, and these fragments formed new buds. This is a possible explanation of the present results although every effort was made to remove the entire bud intact. In these experiments all the buds removed were at least in the closed sphere stage and many were quite large and visibly separated from the parent though still attached by the stalk. It would take considerable powers of regeneration for fragments of such buds to form a whole new zooid.

The 17 buds whose origin it was impossible to determine might possibly be cases of vascular budding (Oka and Watanabe, 1957). They arose during or after the regression of the adult zooids, so a vascular origin seems likely. They were not observed, however, until they were large enough to obscure their point of origin.

Although development of ascidians from egg to tadpole is determinate (Conklin, 1905), the adults have remarkable powers of regeneration (Berrill, 1951). Zhinken (1939) has shown that while tadpoles have little ability to replace lost parts, the oozooid has acquired considerable regulative powers. The present study would indicate that buds also have the ability to regenerate lost tissues from the earliest stages onward.

Berrill has shown (1941b, 1941c, 1945) that the size of the maximum disc and sphere stages increases with succeeding generations and that the size of the adult zooid is clearly related to the number of cells or the diameter of the maximum disc and sphere. This might suggest that the parent determines the size of the new zooid by the number of cells which are initially incorporated into the early stages of the bud. If this were true, then destroying some of these cells would have resulted in smaller adult zooids. However, using length as an index of zooid size, it was found that there was no decrease in size of the zooids damaged at the hemisphere stage. After a bud was damaged it either disappeared completely or reached the predetermined size. Thus the size of the adult does not appear to depend directly on the number of cells originally present since these cells can be replaced. Either the

parent zooid retains control over the growth of the bud or the bud has "received instructions" as to the size it should attain and follows them by regenerating lost tissue and then continuing to grow. Sabbadin (1956c) has shown that zooids with the position of the digestive tube reversed may appear if the growth of the bud is delayed at an early stage. The buds on these abnormal zooids showed a marked tendency to be the same as their parents unless the parent has started to regress before organogenesis is complete. This would indicate that the parents do retain control over the growth and organogenesis of their buds.

Summary

1. The degeneration of the adult zooid of *Botryllus schlosseri*, which normally occurs when the buds become functional, occurred even after all buds were removed.

2. All stages of the growing buds of Botryllus have considerable regenerative ability.

3. Buds damaged in the hemisphere stage became adult zooids with the same length as undamaged zooids of the same age. Control over the size of the adult zooid appears to be maintained during the growth of the bud.

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